

2017

# Differential Responses of Soil Greenhouse Gas Production and Denitrification to Salinity Alterations Along a Wetland Salinity Gradient

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DIFFERENTIAL RESPONSES OF SOIL GREENHOUSE GAS PRODUCTION AND  
DENITRIFICATION TO SALINITY ALTERATIONS ALONG A WETLAND SALINITY  
GRADIENT

A Thesis

Submitted to the Graduate Faculty of the  
Louisiana State University and  
Agriculture and Mechanical College  
in partial fulfillment of the  
requirements for the degree of  
Master of Science

in

The Department of Oceanography and Coastal Sciences

by  
Natalie A. Ceresnak  
B.S., University of Scranton, 2015  
August 2017

## **ACKNOWLEDGEMENTS**

This research is made possible by a grant to Dr. Brian Roberts from the Gulf of Mexico Research Initiative (GoMRI) to the Coastal Waters Consortium II (CWC-II). I would like to thank the Coastal Protection and Restoration Authority (CPRA) for their support through the Coastal Science Assistantship Program (CSAP). I would also like to thank Louisiana Universities Marine Consortium (LUMCON) for providing resources and support.

I am grateful for my co-advisors, Dr. Brian Roberts and Dr. R. Eugene Turner for allowing me the opportunity to complete this research. Their knowledge, guidance and encouragement has helped tremendously through this research process. I am also grateful for my committee member, Dr. Tracy Quirk, and collaborators Dr. Ariella Chelsky and Dr. Troy Hill for their time, insight, and suggestions. Many thanks to my amazing fellow lab members the Lab of Ecosystem Ecology and Biogeochemistry at LUMCON: Samantha Setta, Jacqui Levy, Ron Schuermann, Anthony Rietl, Ekaterina Bulygina, Brendan Kelly and Caleb Bourgeois for their extensive help in the field and lab. Last, but certainly not least, thank you to my family and friends. I am grateful for their support and friendship.

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## ABSTRACT

Coastal wetlands provide several valuable services, such as carbon (C) storage and nitrogen (N) removal. Although wetlands serve as net C sinks, wetland soils release greenhouse gases (GHGs) including carbon dioxide ( $\text{CO}_2$ ), methane ( $\text{CH}_4$ ), and nitrous oxide ( $\text{N}_2\text{O}$ ). Wetlands can buffer the influx of nitrate ( $\text{NO}_3^-$ ) by transforming it into gaseous N ( $\text{N}_2\text{O}$ ,  $\text{N}_2$ ) through denitrification microbial pathway. Salinity is a regulator of soil biogeochemistry and long- (e.g. saltwater intrusion) and short-term (e.g. storm surges, river diversions) exposures may affect soil GHG production and denitrification. In this study, soil GHG production and denitrification enzyme activity (DEA) rates were examined over the course of a growing season (May, July, October) in soils from a freshwater, intermediate, brackish, and saline marsh. The response of GHG production and DEA rates were determined both under ambient and altered salinities (0, 10, 20, 30 psu). Soil  $\text{CO}_2$  and  $\text{CH}_4$  production rates decreased by 83% and >99%, respectively from the freshwater to saline marsh at ambient salinity. Soil  $\text{N}_2\text{O}$  production rates did not vary across marshes, whereas, DEA was highest in May in the intermediate and brackish marshes. Short-term salinity exposure increased soil  $\text{CO}_2$  production in May and October, however, in July, soils displayed lower quality organic matter (high soil C:N), constraining respiration rates. Short-term salinity exposure decreased  $\text{CH}_4$  production, but increased  $\text{N}_2\text{O}$  production in all months. Soil DEA displayed minor decreases with short-term salinity exposure. Soil GHG production in low salinity marshes (e.g. freshwater) had stronger responses to short-term salinity exposure than high salinity marshes (e.g. saline). Collectively, these results indicate that GHG and DEA rates do not always show the same responses to long-term salinity exposure,

which results in shifts of vegetation structures, microbial communities, and soil properties compared to short-term salinity exposure. Sustained shifts to fresher conditions along salinity gradients may increase soil CO<sub>2</sub> and CH<sub>4</sub> production and short-term salinity exposure may increase CH<sub>4</sub> production, but decrease soil CO<sub>2</sub> and N<sub>2</sub>O production. Restoration activities (i.e. river diversions) that consider the interactive effect of salinity on C and N cycling can help reduce GHG footprint and increase nutrient buffering capacities of coastal wetlands.

## 1. INTRODUCTION

Coastal wetlands provide several ecosystem services, such as storm surge protection, water purification, wildlife and fisheries habitat, carbon (C) storage, and nitrogen (N) removal (Craft et al. 2009; Barbier et al. 2011; Engle 2011). Wetlands are highly productive ecosystems and their soils contain 45-75% of all terrestrial organic C (Mitra et al. 2005) despite occupying only 4-6% of earth's land cover (Matthews and Fung 1987; Aselmann and Crutzen 1989). Situated between marine and terrestrial systems, coastal wetlands can remove inputs of N before it reaches aquatic systems (Seitzinger 1988). For example, Louisiana coastal wetlands can remove the influx of inorganic N from agricultural runoff before it reaches the Gulf of Mexico (GoM), which would otherwise contribute to eutrophication and seasonal hypoxia (Rabalais et al. 2002; Turner et al. 2008).

The global area of coastal wetlands has been decreasing at a rate of 1-2% year<sup>-1</sup> since the 1800s (Bridgham et al. 2006; Duarte et al. 2008). Coastal Louisiana contains approximately 37% of herbaceous marsh in the conterminous United States (Couvillion et al. 2011). However, coastal Louisiana has one of the highest rates of wetland loss in the world where approximately 25% (equivalent to 4900 km<sup>2</sup>) of coastal marshes have been lost from 1932 to 2010 (Couvillion et al. 2011). Land loss in coastal Louisiana is due to multiple stressors including relative sea level rise and land subsidence which is enhanced by anthropogenic changes, such as the dredging of canals and resulting spoil banks (Turner 1997), exclusion of sediments from the Mississippi River (Day et al. 2000), and hydrocarbon extraction (Morton et al. 2006). In addition to the loss of land,

these mechanisms can also result in sustained increases in salinity further inland, termed saltwater intrusion (Salinas et al. 1986; Herbert et al. 2015).

Mississippi River diversions have been implemented and proposed to reconnect the river to coastal wetlands to offset saltwater intrusion and rebuild land (CPRA 2017). For example, the Davis Pond Freshwater Diversion began operation in 2002 (maximum design discharge rate of  $300 \text{ m}^3 \text{ s}^{-1}$ ), discharging into the Barataria Basin in southeastern, Louisiana. Diversions introduce fresh, sediment-laden, and nutrient rich water into receiving coastal wetlands (Allison and Meselhe 2010; Allison et al. 2014; CPRA 2017), altering salinity gradients and potentially soil biogeochemical process rates.

Freshwater marsh soil properties (e.g. low salinity, high organic matter, C and N content; Craft 2007; Craft et al. 2009; Wieski et al. 2010) favor high  $\text{CO}_2$  and  $\text{CH}_4$  fluxes (Smith et al. 1983; DeLaune et al. 1983; Poffenbarger et al. 2011), and denitrification rates (Dodla et al. 2008) compared to saline marshes. Long-term salinity regimes structure vegetation and soil properties, regulating organic matter (OM) availability and microbial communities. For example, long-term exposure to increasing salinity may constrain soil respiration (i.e.  $\text{CO}_2$  production) by decreased soil C availability and increased OM recalcitrance, whereas short-term exposure of salinity into freshwater wetland soils may increase soil C carbon loss to the atmosphere, possibly due to increased sulfate ( $\text{SO}_4^{2-}$ ) reduction, thereby increasing  $\text{CO}_2$  emissions (Chambers et al. 2011; Chambers et al. 2013; Neubauer et al. 2013). Sulfate in saltwater can function as an alternative electron acceptor during anaerobic microbial respiration and inhibit methanogenesis, resulting in declines of  $\text{CH}_4$  emissions with salinity (Bartlett et al.

1987; Poffenbarger et al. 2011). Nitrous oxide emissions from wetland soils are typically low, and are released into the atmosphere through two microbial pathways: denitrification (Knowles 1982) and nitrification (Yoshida and Alexander 1970).

Denitrification is a significant removal pathway of bioavailable nitrogen from the soil to the atmosphere (Mitsch and Gosselink 2007), that is facilitated by bacteria under anoxic conditions and transforms nitrate ( $\text{NO}_3^-$ ) and nitrite ( $\text{NO}_2^-$ ) into nitric oxide (NO), nitrous oxide ( $\text{N}_2\text{O}$ ), or molecular nitrogen ( $\text{N}_2$ ) (Seitzinger 1988). Some soil conditions, such as low temperature, low pH, alternating wet and dry cycles, and  $\text{O}_2$  availability can lead to incomplete denitrification and, therefore,  $\text{N}_2\text{O}$  release (Knowles 1982). Salinity can interfere with N cycling by increasing hydrogen sulfide ( $\text{H}_2\text{S}$ ) accumulation, inhibiting the reduction of  $\text{N}_2\text{O}$  into  $\text{N}_2$  (Sørensen et al. 1980) and suppressing nitrification and denitrification (Joye and Hollibaugh 1995; Osborne et al. 2015).

It is important to trace soil  $\text{CH}_4$  and  $\text{N}_2\text{O}$  emissions because they are potent greenhouse gases (GHGs) that have approximately 25 and 298 times greater warming potential, respectively, than  $\text{CO}_2$  on a mole basis for a 100-year time frame (Forster et al. 2007). Coastal wetlands in Louisiana are the last potential sink for terrestrially derived reactive N before they enter the GoM, and so the quantification of N removal to the atmosphere (e.g. denitrification rates) is an important factor to measure.

Long-term salinity exposure can lead to sustained shifts in salinity regimes that alter vegetation composition, associated microbial communities, and soil properties. Short-term salinity exposure may directly increase ionic strength,  $\text{SO}_4^{2-}$  availability, and accumulation of  $\text{H}_2\text{S}$ . Salinity exposure at different timescales (long- and short-term) may affect soil  $\text{CO}_2$ ,  $\text{CH}_4$ ,  $\text{N}_2\text{O}$  production, and denitrification rates differently, but limited

data are available. Here, I describe the results of experiments measuring marsh soil GHG ( $\text{CO}_2$ ,  $\text{CH}_4$ ,  $\text{N}_2\text{O}$ ) production and denitrification potential rates at four marsh sites along a salinity gradient under ambient (to assess long-term salinity regime) and altered salinity conditions (to assess short-term exposure to salinity) during laboratory incubation experiments. The temporal gas emission patterns were examined by repeating measurements on three occasions over one growing season (May, July, and October 2016). It was hypothesized that: (1) soil GHG production and denitrification rates decrease along the ambient salinity gradient from the freshwater to saline marsh; (2) short-term exposure to salinity increases soil  $\text{CO}_2$  production and decreases soil  $\text{CH}_4$  production,  $\text{N}_2\text{O}$  production and denitrification; (2a) low salinity marsh sites have a greater response to short-term exposure to salinity, which may be explained by soil properties; and (3) rates are highest in July because of an increase in microbial activity with higher temperature.

## 2. MATERIALS AND METHODS

### 2.1. Site descriptions

This study took place along a wetland salinity gradient (approximately 110 km transect) in Barataria Basin in southeastern Louisiana, USA. The sampling sites included four vegetation classes (Figure 1; Sasser et al. 2014). The freshwater marsh (29°46'24"N, 90°15'02"W) was located on the northwestern edge Lake Salvador and was the most diverse site, largely dominated by *Sagittaria lancifolia*. The intermediate (29°31'14"N, 90°13'29"W) and brackish (29°26'47"N, 90°05' 52"W) marshes was located along the western and southern edges of South Little Lake (along Bay L'Ours and near Coffee Bayou, respectively) and had greatest biomass of *Bolboschoneus robustus* or *Spartina patens* and *S. patens*/*S. alterniflora*, respectively. The saline *S. alterniflora* marsh site (29°16'48"N, 89°58'28"W) was located on the northern side of Beauregard Island in Barataria Bay. From 1932 to 2011, the land area in Barataria Basin has decreased from 3833 km<sup>2</sup> to 2653 km<sup>2</sup> (equivalent to 30.8% loss) (Couvillion et al. 2011). The Davis Pond freshwater diversion (maximum discharge capacity of 300 m<sup>3</sup> s<sup>-1</sup>) began operation in 2002 and diverts Mississippi River water into the northern region of the Barataria estuary (triangle in square, Figure 1). The surface water salinities in the upper reaches of the Barataria estuary (e.g. Lake Catatouche, Lake Salvador) are near 0 psu. The southernmost reaches of the estuary are connected to the Gulf of Mexico through four tidal passes (Barataria, Camindada, Abel and Quatre Bayou) where surface water salinities range from 5 psu to 35 psu with a mean salinity at 14.6 psu (USGS gauging station: Barataria Pass, Grand Isle, LA 2008-2017). A sediment diversion meant to mitigate land loss is proposed for the mid-Barataria region (triangle

in circle, Figure 1) will introduce sediment, freshwater and nutrients into the basin with a maximum discharge capacity of  $2124 \text{ m}^3 \text{ s}^{-1}$  (CPRA 2017).

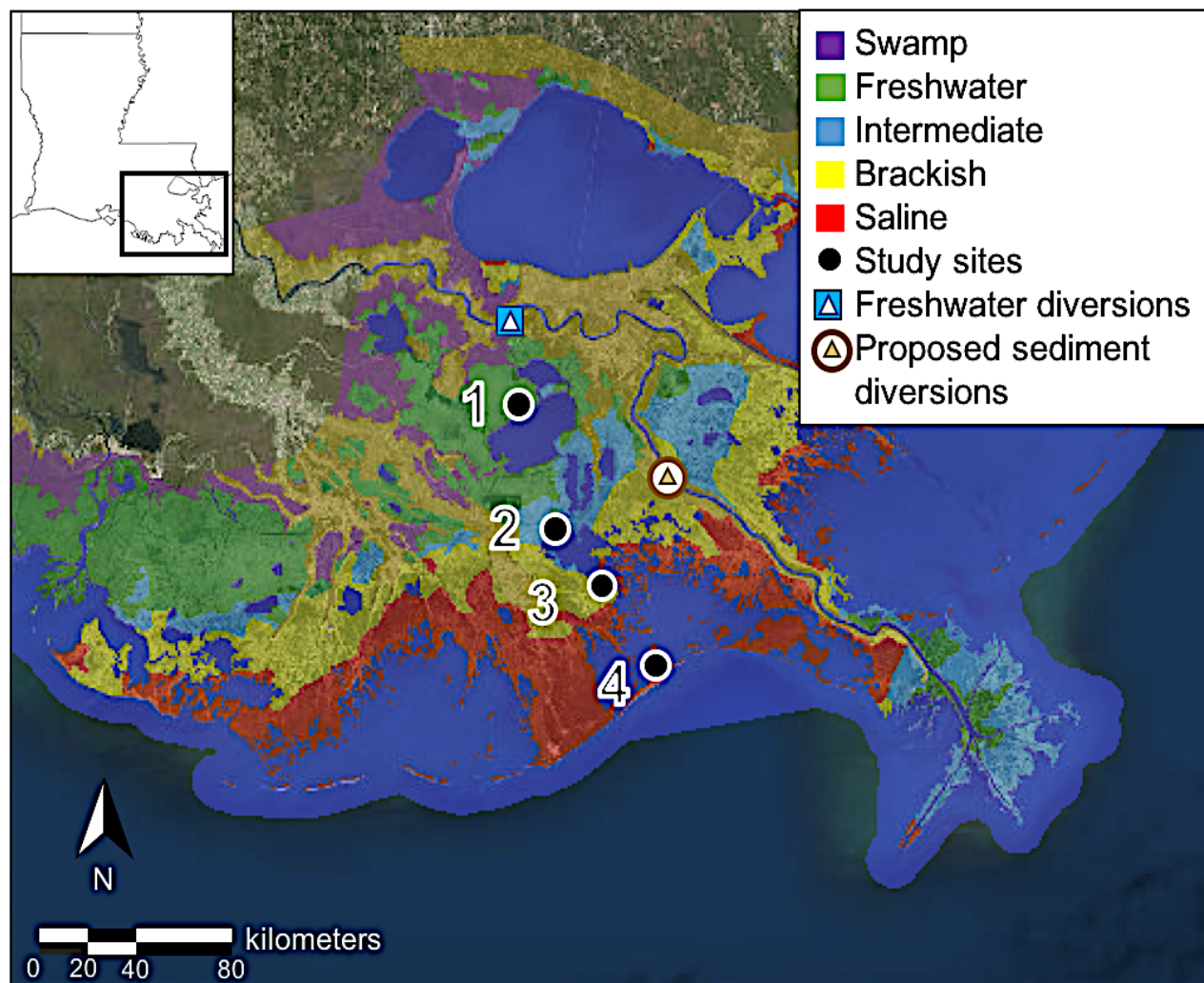


Figure 1. Study sites along Barataria salinity gradient in southeastern Louisiana, USA, including a freshwater (1), intermediate (2), brackish (3) and saline marsh (4) (Sasser et al. 2014). Marsh types are defined by vegetation classification data (2013) in the Coastal Reference Monitoring System (CRMS) based on Sasser et al. 2014. Davis Pond freshwater diversion (maximum design discharge rate of  $300 \text{ m}^3 \text{ s}^{-1}$ ) is located North of study site 1 and proposed sediment diversion (maximum discharge capacity of  $2124 \text{ m}^3 \text{ s}^{-1}$ ) will be in the mid-Barataria estuary.

## **2.2. Experimental design and field sampling**

The four marsh sites were sampled on three separate occasions in May, July, and October 2016. At each site, a post was established at the marsh edge and another post was positioned 20 m (5 m for saline marsh site) from the marsh edge into the marsh interior. Three plots (0.25 m x 0.25 m) were positioned a random distance (1, 2, 3, 4, or 5 m) from the interior post at a random angle (0° to 90°) at each site. A different random distance and angle were chosen on each sampling occasion. The plots within each site were oriented equidistant apart from one another at a 120° angle.

The litter from each plot was collected and stored in plastic bags. The aboveground biomass was clipped at the base of plants at the sediment surface and stored in separate plastic bags. The belowground biomass was collected using a metal auger corer (6 cm diameter x 30 cm depth) with beveled edges (AMS part #53764). Duplicate surface soil cores (6.9 cm diameter x 5 cm depth) were collected using an acrylic core with beveled edges and stored in Whirl-Packs. Duplicate 5 cm deep porewater cores were collected using a 2.6 cm diameter syringe corer and stored in centrifuge tubes. Separate surface (0.5 cm) cores were collected in duplicate using a 1.5 cm syringe corer with and stored in centrifuge tubes for determination of benthic microalgal abundances. Soil redox potentials were measured using Thermo Scientific Orion redox/ORP electrode (model 9678BNWP), and surface soil temperatures were measured. During October sampling, intact soil cores were collected in 2 plots per site (n=8) using polyvinyl chloride (PVC) cores (9.5 cm x 30 cm depth) to measure porewater hydrogen sulfide (H<sub>2</sub>S) concentrations. The overlying water (adjacent baywater if overlying was not present) was placed in cores to prevent oxidation of soils,

the bottom of the cores were capped, and tops were sealed using Parafilm® to prevent spills. Adjacent bay water and overlying water at plots (if present) were collected in amber high-density polyethylene (HDPE) bottles and salinity, temperature, conductivity and total dissolved solids (TDS) were measured using a YSI Professional instrument (Pro30) handheld probe. All soil and water samples were kept on ice in the field until return to the laboratory.

### **2.3. Soil and water properties**

Once at the laboratory, surface soil cores were weighed wet, then subsampled to determine water content by drying at 80°C until constant mass. Bulk density was calculated as the dry mass of the core divided by the core volume. Approximately 5.0 g of field-moist soil were added to 50 mL centrifuge tubes with 30 mL of 2N KCl to extract samples for determination of NO<sub>3</sub>-N and NH<sub>4</sub>-N concentrations. Samples were shaken at 250 rpm for two hours, centrifuged, and filtered through 0.20 µm syringe filters (Corning, #431224, Corning, NY). Phosphate was extracted from field-moist soil by adding ~5.0 g of soil and 30 mL of 0.5 M NaHCO<sub>3</sub> to 50 mL centrifuge tubes, shaking for 16 hours, centrifuging, and filtering through 0.20 µm syringe filters. All extractable nutrient samples were stored frozen until analysis (see below). The remaining soil was dried, ground with a mortar and pestle, and passed through a 2 mm mesh sieve. Sub-samples (~20 g) were placed in aluminum weight boats and combusted at 500°C for four hours and the change in mass was used to calculate soil organic matter content. Additional sub-samples were placed into 20 mL glass scintillation vials, stored in a glass desiccator, and fumigated with concentrated HCl vapors for 72 hours to remove carbonates. Samples were then analyzed for total organic C and total N using a Flash

1200 Elemental Analyzer (CE Elantech, Lakewood, New Jersey). Sediment standards (National Institute of Standards and Technology, Buffalo River Sediment, 2704) were run concurrently, which yielded organic C recoveries of  $100.9 \pm 0.6\%$ ,  $100.2 \pm 1.8\%$ , and  $101.7 \pm 1.6\%$  in May, July, and October, respectively. Total P was extracted by combusting 0.2 g soil samples with 0.5 mL of a 50% (w/v) solution of  $\text{Mg}(\text{NO}_3)_2$  at  $550^\circ\text{C}$  for 1.5 hours and then shaking for 16 hours with 10% HCl. The supernatant was then analyzed for  $\text{PO}_4\text{-P}$  concentrations as described below. The sediment standards for determination of total P concentration (National Institute of Standards and Technology, Estuarine Sediment, 1646a) were digested and analyzed concurrently with samples and yielded a mean recovery of  $113.7 \pm 0.9\%$ ,  $101.0 \pm 0.7\%$ , and  $97.9 \pm 0.05\%$  in May, July, and October, respectively. Soil molar C:N and N:P ratios were calculated from the C, N, and P concentrations. The soil pH was measured using a 1:1 soil:water ratio with a Thermo Scientific Orion 3-Star pH meter equipped with an Orion ROSS Ultra Triode pH/ATC probe. Porewater cores were centrifuged (3000 rpm) and the salinity of the supernatant was measured using a refractometer. Bay and plot water samples were filtered through acid-cleaned (10% HCl),  $0.2\ \mu\text{m}$  pore size membrane filters (Pall Supor® 200) under low vacuum pressure, stored frozen until analysis, and analyzed concurrently with the determination of extractable nutrients.

The concentrations of inorganic hydrogen sulfide ( $\text{H}_2\text{S}$ ) in porewater were determined in intact soil cores collected in October. Intact cores were placed directly into an Ultra High Purity (UHP)  $\text{N}_2$  flushed glove bag upon return to the laboratory to ensure that the soils were not oxidized. Intact soil cores were extruded and a soil subsample (26 mm diameter x 5 cm depth) was collected. The samples were capped,

centrifuged (3000 rpm) to extract porewater, and returned to the glove bag where a 250  $\mu$ l subsample of the porewater was added to 6 mL of 2 % zinc acetate (ZnAc) in scintillation vials. Dye solution (3.728 g n,n-dimethyl-p-phenylene diamine mono hydrochloride 6.0 g  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ) was then added (5 mL) and the tube was quickly capped. Blank samples were made with the reagents described above, but with 250  $\mu$ l of NANOpure water instead of a porewater sample. All samples were shaken and kept in dark for at least 0.5 hours, but for no more than 2 hours. Direct spectrophotometric determinations of  $\text{H}_2\text{S}$  concentrations were determined by analyzing porewater solutions on a Thermo Finnigan UNICAM UV 300 spectrophotometer set at 670 nm. Porewater  $\text{H}_2\text{S}$  concentrations were calculated as the difference between sample absorbance and blank absorbance, and dividing by a constant (0.542) that was empirically determined by a method adapted from Gilboa-Garber (1971).

Water and extractable nutrient ( $\text{NO}_3^- + \text{NO}_2^-$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$ ) samples were analyzed using a Lachat Instruments QuickChem® FIA + 8000 Series Automated Ion Analyzer with an ASX-400 Series XYZ Autosampler. Samples were analyzed simultaneously for dissolved  $\text{NO}_3^- + \text{NO}_2^-$  (by Cu-Cd reduction followed by azo colorimetry) and  $\text{PO}_4^{3-}$  (by the automated ascorbic acid reduction method) but were analyzed separately for dissolved  $\text{NH}_4^+$  (by phenate colorimetry) to prevent contamination of the samples by fumes from the  $\text{NH}_4\text{Cl}$  buffer used in the analysis for  $\text{NO}_3^- + \text{NO}_2^-$  (American Public Health Association, 1992). Standard curves were prepared using standard  $\text{PO}_4\text{-P}$ ,  $\text{NO}_3\text{-N}$ , and  $\text{NH}_4\text{-N}$  stock solutions (Hach, Loveland CO) and yielded  $r^2$  values of  $\geq 0.99$ .

## **2.4. Vegetation**

The litter from each plot was dried until constant weight at 70°C. Aboveground live biomass was separated by species and considered live if the plant contained green tissues, indicating photosynthetic activity. The remaining aboveground dead biomass, and species-separated live biomasses were dried until constant weight at 70°C. The belowground cores were rinsed to wash away soil, separated by live and dead biomasses (total roots + rhizomes) and dried until constant weight at 70°C. Live roots and rhizomes were white and turgid, and dead roots and rhizomes were dark and flaccid. Benthic microalgal abundances were extracted by adding 20 mL of 90 % acetone to cores and freezing for 24 to 48 hours. The samples were brought to room temperature, vortexed, and a 1 mL of supernatant was added to tubes containing 5 mL of 90 % acetone. Samples were measured on the Turner Designs 10-AU Fluorometer before (to measure chlorophyll *a*) and after 4 drops of 10% HCl was added (to measure phaeopigments). Chlorophyll *a* and phaeopigment concentrations were expressed in terms of dry soil per unit surface area.

## **2.5. Biogeochemical processes**

### **2.5.1. Greenhouse Gas (GHG) Production**

Greenhouse gas (CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O) production were measured using anaerobic soil slurries. Twenty grams of field-moist soil and 40 mL of filtered salinity-adjusted water were added to and homogenized in 125 mL flasks. Salinity solutions were made to achieve 5 target salinity treatments: ambient porewater, 0, 10, 20, and 30 psu. The freshwater marsh site included a 5 psu treatment because the ambient salinity was 0 psu. Triplicate flasks per treatment were capped with Suba Seal® stoppers (#49)

and flushed with ultra-high purity N<sub>2</sub> for 10 minutes. A 10 mL headspace sample was collected after 1, 24, 48, 72, and 96 hours and then stored in N<sub>2</sub>-flushed 5.9 mL Exetainers®. Prior to sampling, each flask was vigorously shaken to equilibrate gases between the slurry and headspace. Ten mL of N<sub>2</sub> were added to each flask after sample collection to maintain a constant pressure. Gas samples were analyzed on a gas chromatograph (Shimadzu GC-2014, Shimadzu, Columbia, MD) equipped with a <sup>63</sup>Ni electron capture detector, a methanizer, and a flame-ionization detector. All gas concentrations were corrected for dissolved gases using Bunsen coefficients and for dilution through multiple samplings. The corrected concentrations were regressed against time and the linear portions of the accumulation curves were used to estimate production rates. All rates were expressed in terms of dry soil.

### **2.5.2. Denitrification Enzyme Activity (DEA)**

Denitrification potentials were determined by measuring denitrification enzyme activity (DEA) using a modification of the acetylene (C<sub>2</sub>H<sub>2</sub>) inhibition technique which prevents the reduction of N<sub>2</sub>O to N<sub>2</sub> (Groffman et al. 1999). Twenty grams of field-moist soil and 40 mL of filtered salinity-adjusted water were homogenized in 125-mL flasks. The salinity solutions were made to achieve 5 target salinity treatments: ambient porewater, 0, 10, 20, and 30 psu; the fresh marsh site included a 5 psu treatment since ambient salinity was 0 psu. Excess carbon (C), nitrogen (N) and phosphorus (P) were added to the flasks with target concentrations of 300 mg C, 50 mg N and 100 mg P reached by adding D-glucose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>), potassium nitrate (KNO<sub>3</sub>) and potassium phosphate monobasic (KH<sub>2</sub>PO<sub>4</sub>). Triplicate flasks per treatment were capped with Suba Seal® stoppers (#49) and flushed with ultra-high purity N<sub>2</sub> for 10 minutes. Ten mL

acetylene ( $C_2H_2$ ) were added to each flask to inhibit the reduction of  $N_2O$  to  $N_2$ . The flasks were shaken at 250 rpm in between sampling points. A 10 mL headspace sample was collected after 0.5, 1, 2, and 4 hours and stored in  $N_2$ -flushed 5.9 mL Exetainers®. Nine mL of  $N_2$  and 1 mL  $C_2H_2$  were added to each flask after sample collection to maintain a constant pressure. Gas samples were analyzed on a gas chromatograph (Shimadzu GC-2014, Shimadzu, Columbia, MD) equipped with a  $^{63}Ni$  electron capture detector. All gas concentrations were corrected for dissolved gases using Bunsen coefficients and for dilution through multiple samplings. Corrected concentrations were regressed against time and the linear portions of the accumulation curves were used to estimate production rates. All rates were expressed in terms of dry soil.

## **2.6. Statistical analysis**

All data were tested for normality using Shapiro-Wilks and homogeneity of variance using Levene's test. When necessary, data were natural-log transformed. To evaluate differences in soil properties at different marsh sites (freshwater, intermediate, brackish and saline;  $n=4$ ), a one-way analysis of variance (ANOVA) was used and significant differences were determined using Tukey's Honestly Significant Difference (HSD) test. To test variations in soil properties across marsh sites and months, a principal component analysis (PCA) was run. To evaluate GHG ( $CO_2$ ,  $CH_4$  and  $N_2O$ ) production and denitrification potentials (DEA) at ambient salinities, a two-way ANOVA with main effects based on marsh site (freshwater, intermediate, brackish, and saline;  $n=4$ ) and month (May, July, and October;  $n=3$ ) was run. Significant differences were determined using Tukey's HSD test. A PCA was further used to analyze the relationship

between soil properties and ambient rates by conducting linear regression analyses between soil PCA dimensions (1 and 2) and ambient rates. The effects of short term salinity alterations at different marsh sites on GHG production and DEA rates were tested using a two-way analysis of covariance (ANCOVA; Appendix A, Table A-3 and A-4) with salinity (continuous covariate) and marsh site (categorical;  $n=4$ ) as variables. Linear regression analyses were run to determine the slopes of GHG production and DEA rates in response to salinity at each marsh site in each month (total of 48; Appendix A, Table A-5). Pairwise comparisons were made to determine significant differences between slopes. The slope of  $\text{CO}_2$  production versus the salinity was determined by calculating the individual linear regressions at each marsh site and month ( $n=12$ ; Table 13) and observing slopes. Relationships between the observed  $\text{CO}_2$  slope ( $\text{nmol gdw}^{-1} \text{ day}^{-1} \text{ psu}^{-1}$ ) and soil properties (e.g. organic carbon) were determined by regression analysis. All statistical analyses were conducted at  $\alpha=0.05$  using the statistical software R.

### 3. RESULTS

#### 3.1. Site characteristics

##### 3.1.1. Vegetation patterns

The species richness declined with increased ambient salinity. The freshwater and intermediate marshes had significantly higher richness ( $5.3 \pm 0.7$  and  $4.7 \pm 0.7$  species per plot, respectively) than the brackish (2) and saline (1) marsh sites (ANOVA;  $p < 0.001$ ; Table 1). When averaged across marsh sites, *Sagittaria lancifolia* ( $68.9 \pm 9.4\%$ ) contributed more than half to the total freshwater marsh biomass. *Bolboschoneus robustus* ( $40.5 \pm 9.7\%$ ) contributed the most to total intermediate marsh biomass, except in July, where *Spartina patens* contributed the most ( $43.5 \pm 8.4\%$ ). The brackish marsh was co-dominated by *S. alterniflora* and *S. patens*, whereas the saline marsh was homogenously covered by *S. alterniflora*. Vegetation biomass patterns were not different across marsh sites or months. The mean ( $\pm$  SE) aboveground live biomass was  $1,052 \pm 90 \text{ g m}^{-2}$ , aboveground dead biomass was  $408 \pm 68 \text{ g m}^{-2}$ , belowground live biomass (roots + rhizomes at 30cm depth) was  $1,228 \pm 216 \text{ g m}^{-2}$ , and belowground dead biomass (roots + rhizomes at 30 cm depth) was  $284 \pm 32 \text{ g m}^{-2}$ . The benthic microalgal abundances chlorophyll a (ANOVA;  $p = 0.144$ ) and phaeopigments ( $p = 0.302$ ) did not vary across marsh sites. Although the vegetation biomass patterns were not significantly different across marsh sites, vegetation composition may have influenced differences in soil properties.

Table 1. Aboveground biomass and belowground biomass (30 cm depth) at each marsh site (freshwater, intermediate, brackish and saline) during each month (May, July, and October 2016). Means ( $\pm$  standard error) were calculated for each marsh site. Litter was not collected or quantified in May. Fresh=freshwater, inter=intermediate, brack=brackish.

Marsh site	Chlorophyll a ( $\mu\text{g cm}^{-2}$ )	Phaeo-pigments ( $\mu\text{g cm}^{-2}$ )	Species richness (species per plot)	Aboveground live biomass ( $\text{g m}^{-2}$ )	Aboveground dead biomass ( $\text{g m}^{-2}$ )	Litter ( $\text{g m}^{-2}$ )	Belowground live biomass ( $\text{g m}^{-2}$ )	Belowground dead biomass ( $\text{g m}^{-2}$ )
May								
Fresh	1.9 $\pm$ 1	5.6 $\pm$ 3.2	5.3 $\pm$ 0.7	1430 $\pm$ 44.8	104 $\pm$ 46.4	-	1,242 $\pm$ 40	196 $\pm$ 6.1
Inter	2.8 $\pm$ 1.3	5.6 $\pm$ 2.7	5.7 $\pm$ 0.9	912 $\pm$ 17.2	238 $\pm$ 106	-	1,672 $\pm$ 80	497 $\pm$ 27
Brack	3.2 $\pm$ 1.6	4.2 $\pm$ 2.2	2	1474 $\pm$ 40.9	847 $\pm$ 189	-	961 $\pm$ 27	321 $\pm$ 12
Saline	3.9 $\pm$ 1.1	6.3 $\pm$ 1.1	1	1221 $\pm$ 43.8	394 $\pm$ 21	-	911 $\pm$ 52	91 $\pm$ 5.6
July								
Fresh	2.4 $\pm$ 2.2	9.4 $\pm$ 2.9	5.3 $\pm$ 0.3	738 $\pm$ 24.2	355 $\pm$ 113	53.1 $\pm$ 2.4	2,195 $\pm$ 76	160 $\pm$ 6.5
Inter	1.4 $\pm$ 1.1	8.7 $\pm$ 4.2	3.7 $\pm$ 0.3	605 $\pm$ 14.1	186 $\pm$ 23.0	48.3 $\pm$ 12.3	835 $\pm$ 41	561 $\pm$ 34
Brack	3.5 $\pm$ 2.3	5.2 $\pm$ 3.3	2	829 $\pm$ 23.6	770 $\pm$ 267	102 $\pm$ 30.2	1,057 $\pm$ 21	266 $\pm$ 12
Saline	2.2 $\pm$ 1	5.9 $\pm$ 1.5	1	1126 $\pm$ 41.2	369 $\pm$ 44.4	53.6 $\pm$ 14.7	1,687 $\pm$ 90	60 $\pm$ 3.5
October								
Fresh	0.7 $\pm$ 0.3	5.8 $\pm$ 3.5	5 $\pm$ 1.2	712 $\pm$ 20.9	456 $\pm$ 274	110 $\pm$ 53.5	1,388 $\pm$ 67	219 $\pm$ 7.8
Inter	5.7 $\pm$ 3.6	7.4 $\pm$ 2.4	4.7 $\pm$ 0.9	853 $\pm$ 23.0	346 $\pm$ 145	136 $\pm$ 67.0	754 $\pm$ 44	449 $\pm$ 27
Brack	6.3 $\pm$ 5.2	5.2 $\pm$ 4.4	2	1394 $\pm$ 36.9	641 $\pm$ 136	79.5 $\pm$ 12.7	918 $\pm$ 28	380 $\pm$ 10
Saline	6.0 $\pm$ 3.8	9.1 $\pm$ 2.6	1	1331 $\pm$ 48.5	185 $\pm$ 61.7	105 $\pm$ 17.1	1,117 $\pm$ 57	212 $\pm$ 8.4
Means								
Fresh	1.7 $\pm$ 0.5	6.9 $\pm$ 1.2	5.2 $\pm$ 0.1 <sup>a</sup>	960 $\pm$ 235	305 $\pm$ 105	81 $\pm$ 23	1,608 $\pm$ 296	192 $\pm$ 17
Inter	3.3 $\pm$ 1.3	7.3 $\pm$ 0.9	4.7 $\pm$ 0.6 <sup>a</sup>	790 $\pm$ 94	257 $\pm$ 47	92 $\pm$ 36	1,087 $\pm$ 293	502 $\pm$ 33
Brack	4.4 $\pm$ 1.0	4.9 $\pm$ 0.3	2 <sup>b</sup>	1,232 $\pm$ 203	753 $\pm$ 60	91 $\pm$ 9.1	979 $\pm$ 41	323 $\pm$ 33
Saline	4.0 $\pm$ 1.1	7.1 $\pm$ 1.0	1 <sup>b</sup>	1,226 $\pm$ 60	316 $\pm$ 66	80 $\pm$ 21	1,238 $\pm$ 232	121 $\pm$ 46

### 3.1.2. Soil properties

Spatial patterns (marsh sites) and temporal patterns (months) explained 62% and 13% of the variability in soil properties, respectively (Appendix A, Figure A-1). Because spatial patterns explained most (62%) of the soil property variability, the mean marsh site-level soil properties were calculated by averaging the results across months (Table 2). Freshwater marshes were correlated with a positive PCA dimension 1 soil properties: organic matter, total nitrogen, organic carbon, C:N, extractable  $\text{PO}_4^{3-}$ , total phosphorus and redox potential. Saline marsh soils are correlated with negative PCA dimension 1 properties: bulk density, porewater salinity, and pH (Table 2; Appendix A, Figure A-1). From the freshwater to the saline marsh, the porewater salinity, bulk density, and pH tended to increase. However, the water content, redox, organic matter, organic carbon, total nitrogen, total phosphorus, C:N, and extractable  $\text{PO}_4^{3-}$  decreased from the freshwater to saline marsh ( $p < 0.001$ , Table 2). For example, soil organic carbon in the freshwater marsh was approximately 2 times and 6 to 7 times greater than the intermediate/brackish marsh and saline marsh, respectively. The soil extractable  $\text{NO}_3^-$  ( $p = 0.603$ ) did not vary across marsh sites. The porewater  $\text{H}_2\text{S}$  in October freshwater and intermediate marshes ( $0.15 \pm 0.07 \mu\text{g gdw}^{-1}$ ) was lower than in the brackish and saline marsh soils ( $2.9 \pm 1.1 \mu\text{g gdw}^{-1}$ ;  $p = 0.002$ ). The C:N ratios displayed temporal patterns and was driven by both higher C and lower total N where mean July ( $19 \pm 1.4$ ) values were higher than May ( $15 \pm 0.63$ ) and October ( $16 \pm 0.79$ ). This pattern is due to the high C:N values found in the freshwater ( $23 \pm 3.4$ ), intermediate ( $19 \pm 2.4$ ) and brackish ( $22 \pm 2.3$ ) marsh in July, whereas the C:N ratio in May and October remained at the 16 to 18 range.

Table 2. Soil properties along the salinity gradient at each marsh site (freshwater, intermediate, brackish and saline) in May, July and October 2016. Means ( $\pm$  standard error) were calculated for each marsh site and different lowercase letters represent significant differences between marsh sites using Tukey's HSD test at  $\alpha=0.05$ . Fresh=freshwater, inter=intermediate, brack=brackish.

Marsh site	Porewater salinity (psu)	Bulk density (g cm <sup>-3</sup> )	Water content (%)	Redox (mV)	pH	Organic matter (%)	Organic C (%)	Total N (%)	C:N (mol:mol)	Total P (μmol g <sup>-1</sup> )	Extrac-table NO <sub>3</sub> <sup>-</sup> (μmol g <sup>-1</sup> )	Extrac-table PO <sub>4</sub> <sup>3-</sup> (μmol g <sup>-1</sup> )
May												
Fresh	0	0.10 $\pm$ 0.004	91 $\pm$ 1.1	300 $\pm$ 98	6 $\pm$ 0.06	56 $\pm$ 6.5	30 $\pm$ 1.6	2.1 $\pm$ 0.1	16 $\pm$ 0.2	1,134 $\pm$ 65	0.58 $\pm$ 0.14	3.5 $\pm$ 1.2
Inter	1.3 $\pm$ 0.6	0.18 $\pm$ 0.01	83 $\pm$ 1.3	175 $\pm$ 95	6.2 $\pm$ 0.2	28 $\pm$ 0.8	18 $\pm$ 1.4	1.3 $\pm$ 0.1	16 $\pm$ 0.6	740 $\pm$ 43	0.15 $\pm$ 0.14	1.6 $\pm$ 0.5
Brack	3.3 $\pm$ 0.3	0.23 $\pm$ 0.01	83 $\pm$ 0.6	-76 $\pm$ 14	6.7 $\pm$ 0.3	30 $\pm$ 2.4	18 $\pm$ 0.5	1.2 $\pm$ 0.04	17 $\pm$ 1.1	615 $\pm$ 29	0.30 $\pm$ 0.03	0.5 $\pm$ 0.2
Saline	14	0.49 $\pm$ 0.07	66 $\pm$ 3	-99 $\pm$ 42	6.5 $\pm$ 0.7	11 $\pm$ 0.8	5.2 $\pm$ 0.3	0.51 $\pm$ 0.02	12 $\pm$ 0.3	550 $\pm$ 13	0.15 $\pm$ 0.06	0.2 $\pm$ 0.04
July												
Fresh	0	0.10 $\pm$ 0.01	90 $\pm$ 1.6	208 $\pm$ 28	5.4 $\pm$ 0.3	58 $\pm$ 8.5	34 $\pm$ 1.6	1.8 $\pm$ 0.3	23 $\pm$ 3.4	1,222 $\pm$ 232	0.58 $\pm$ 0.11	2.2 $\pm$ 0.6
Inter	2.2 $\pm$ 0.8	0.17 $\pm$ 0.01	84 $\pm$ 0.6	173 $\pm$ 18	4.9 $\pm$ 0.3	34 $\pm$ 7.2	18 $\pm$ 1.9	1.1 $\pm$ 0.03	19 $\pm$ 2.4	761 $\pm$ 53	0.40 $\pm$ 0.07	0.7 $\pm$ 0.2
Brack	2.5 $\pm$ 0.5	0.21 $\pm$ 0.03	84 $\pm$ 1.6	-296 $\pm$ 21	7.2 $\pm$ 0.3	28 $\pm$ 8.6	20 $\pm$ 3	1 $\pm$ 0.06	22 $\pm$ 2.3	577 $\pm$ 28	0.45 $\pm$ 0.05	0.6 $\pm$ 0.1
Saline	17 $\pm$ 0.5	0.51 $\pm$ 0.21	66 $\pm$ 10	-326 $\pm$ 21	7.6 $\pm$ 0.1	9.5 $\pm$ 2.7	4.8 $\pm$ 1.3	0.44 $\pm$ 0.09	13 $\pm$ 0.9	535 $\pm$ 22	0.22 $\pm$ 0.08	0.2 $\pm$ 0.05
October												
Fresh	0.3 $\pm$ 0.1	0.10 $\pm$ 0.02	92 $\pm$ 1.5	53 $\pm$ 25	5.8 $\pm$ 0.3	76 $\pm$ 0.7	36 $\pm$ 0.5	2.6 $\pm$ 0.2	17 $\pm$ 0.9	1,114 $\pm$ 101	0.20 $\pm$ 0.02	2.6 $\pm$ 0.5
Inter	0.9 $\pm$ 0.9	0.23 $\pm$ 0.06	81 $\pm$ 4.9	-121 $\pm$ 230	5.8 $\pm$ 0.2	36 $\pm$ 15	19 $\pm$ 0.7	1.4 $\pm$ 0.01	16 $\pm$ 0.4	685 $\pm$ 77	0.42 $\pm$ 0.12	0.5 $\pm$ 0.1
Brack	2.3 $\pm$ 0.4	0.19 $\pm$ 0.01	85 $\pm$ 0.9	-276 $\pm$ 10	7.2 $\pm$ 0.2	33 $\pm$ 0.6	17 $\pm$ 1.4	1.1 $\pm$ 0.05	18 $\pm$ 0.8	548 $\pm$ 34	0.33 $\pm$ 0.06	0.5 $\pm$ 0.01
Saline	18 $\pm$ 0.8	0.43 $\pm$ 0.1	68 $\pm$ 4.9	-337 $\pm$ 29	7.5 $\pm$ 0.2	16 $\pm$ 5.1	6.2 $\pm$ 2.0	0.58 $\pm$ 0.08	13 $\pm$ 2.2	526 $\pm$ 47	0.76 $\pm$ 0.22	0.3 $\pm$ 0.03
Means												
Fresh	0.11 $\pm$ 0.1 <sup>c</sup>	0.10 $\pm$ 0.003 <sup>c</sup>	91 $\pm$ 0.5 <sup>a</sup>	187 $\pm$ 72 <sup>a</sup>	5.7 $\pm$ 0.2 <sup>b</sup>	63 $\pm$ 6.3 <sup>a</sup>	34 $\pm$ 1.9 <sup>a</sup>	2.2 $\pm$ 0.2 <sup>a</sup>	19 $\pm$ 2.2 <sup>a</sup>	1,157 $\pm$ 33 <sup>a</sup>	0.46 $\pm$ 0.1	2.8 $\pm$ 0.4 <sup>a</sup>
Inter	1.5 $\pm$ 0.4 <sup>bc</sup>	0.19 $\pm$ 0.02 <sup>b</sup>	83 $\pm$ 0.9 <sup>b</sup>	76 $\pm$ 98 <sup>a</sup>	5.6 $\pm$ 0.4 <sup>b</sup>	33 $\pm$ 2.5 <sup>b</sup>	18 $\pm$ 0.4 <sup>b</sup>	1.3 $\pm$ 0.08 <sup>b</sup>	17 $\pm$ 0.8 <sup>a</sup>	729 $\pm$ 23 <sup>b</sup>	0.33 $\pm$ 0.09	0.9 $\pm$ 0.3 <sup>b</sup>
Brack	2.7 $\pm$ 0.3 <sup>b</sup>	0.21 $\pm$ 0.01 <sup>b</sup>	84 $\pm$ 0.6 <sup>b</sup>	-216 $\pm$ 70 <sup>b</sup>	7.0 $\pm$ 0.2 <sup>a</sup>	30 $\pm$ 1.4 <sup>b</sup>	18 $\pm$ 0.7 <sup>b</sup>	1.1 $\pm$ 0.06 <sup>b</sup>	19 $\pm$ 1.5 <sup>a</sup>	580 $\pm$ 19 <sup>c</sup>	0.36 $\pm$ 0.04	0.5 $\pm$ 0.01 <sup>bc</sup>
Saline	16 $\pm$ 1.4 <sup>a</sup>	0.48 $\pm$ 0.02 <sup>a</sup>	67 $\pm$ 0.8 <sup>c</sup>	-254 $\pm$ 78 <sup>b</sup>	7.2 $\pm$ 0.4 <sup>a</sup>	12 $\pm$ 1.9 <sup>b</sup>	5.4 $\pm$ 0.4 <sup>c</sup>	0.5 $\pm$ 0.04 <sup>c</sup>	12 $\pm$ 0.2 <sup>b</sup>	537 $\pm$ 7.0 <sup>c</sup>	0.38 $\pm$ 0.2	0.2 $\pm$ 0.03 <sup>c</sup>

### 3.2. Greenhouse gas production and denitrification potential along the ambient salinity gradient

The soil CO<sub>2</sub> production rates at ambient salinities were different across marsh sites (ANOVA;  $p < 0.001$ ), where rates decreased with salinity from the freshwater ( $19731 \pm 2038$  nmol gdw<sup>-1</sup> day<sup>-1</sup>), intermediate ( $9169 \pm 1089$  nmol gdw<sup>-1</sup> day<sup>-1</sup>), brackish ( $4889 \pm 977$  nmol gdw<sup>-1</sup> day<sup>-1</sup>) to saline ( $3265 \pm 564$  nmol gdw<sup>-1</sup> day<sup>-1</sup>) marsh soil in all months (Figure 2a; Appendix A, Table A-1). These rates were variable over the course of the growing season ( $p = 0.004$ ), with higher rates in May and July compared to October. Methane production was significantly higher in the freshwater marsh ( $2736 \pm 1352$  nmol gdw<sup>-1</sup> day<sup>-1</sup>) than in the other three marsh sites: intermediate ( $70 \pm 28$  nmol gdw<sup>-1</sup> day<sup>-1</sup>), brackish ( $53 \pm 36$  nmol gdw<sup>-1</sup> day<sup>-1</sup>) and saline ( $3 \pm 2$  nmol gdw<sup>-1</sup> day<sup>-1</sup>) marsh (ANOVA;  $p < 0.001$ ; Figure 2b; Appendix A, Table A-1) with the pattern consistent between months ( $p = 0.50$ ). The ambient salinity soil N<sub>2</sub>O production rates were significantly different across marsh sites (ANOVA;  $p = 0.03$ ; Figure 2c; Appendix A, Table A-1), but there were no significant pairwise differences (Tukey's HSD test,  $p > 0.05$ ). The soil N<sub>2</sub>O production rates were higher in July rates ( $3.4 \pm 1.3$  N<sub>2</sub>O nmol gdw<sup>-1</sup> day<sup>-1</sup>) than May or October ( $0.13 \pm 0.06$  and  $1.0 \pm 0.5$  N<sub>2</sub>O nmol gdw<sup>-1</sup> day<sup>-1</sup>, respectively;  $p < 0.001$ ). The denitrification potentials were different depending on month and marsh sites ( $p = 0.001$ ; Figure 2d; Appendix A, Table A-1) where the highest rates were in May intermediate ( $142 \pm 11$  nmol gdw<sup>-1</sup> day<sup>-1</sup>) and brackish ( $145 \pm 22$  nmol gdw<sup>-1</sup> day<sup>-1</sup>) marshes and lowest rates were in the July freshwater ( $30 \pm 2.6$  nmol gdw<sup>-1</sup> day<sup>-1</sup>) and October brackish ( $40 \pm 4.2$  nmol gdw<sup>-1</sup> day<sup>-1</sup>) marshes.

The marshes are likely net C sinks, but the soils can be sources of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O to the atmosphere, and the relative contribution of each gas varied along the ambient salinity gradient (Appendix A, Figure A-2). The soil radiative forcing trend was similar in all months and tended to decrease from the freshwater (2.0 mg CO<sub>2</sub> equivalents gdw<sup>-1</sup> day<sup>-1</sup>), intermediate (0.72 mg CO<sub>2</sub> equivalents gdw<sup>-1</sup> day<sup>-1</sup>), brackish (0.24 mg CO<sub>2</sub> equivalents gdw<sup>-1</sup> day<sup>-1</sup>), to saline (0.15 mg CO<sub>2</sub> equivalents gdw<sup>-1</sup> day<sup>-1</sup>) marsh. The CH<sub>4</sub> contribution to soil radiative forcing decreases from 55% in the freshwater to 23%, 9%, and 1% in intermediate, brackish, and saline marsh, respectively. The soil CO<sub>2</sub> fluxes account for 44% in the freshwater marsh, but > 69% in all other marsh sites, with N<sub>2</sub>O being a minor contributor across all sites.

Both soil CO<sub>2</sub> and CH<sub>4</sub> production rates increased with marsh site-level soil properties ( $r^2=0.91$ ,  $p<0.001$  and  $r^2=0.85$ ,  $p<0.001$ , respectively; Appendix A, Table A-2): organic matter (%), total nitrogen (%), organic carbon (%), extractable phosphate ( $\mu\text{mol g}^{-1}$ ) total phosphorus ( $\mu\text{mol g}^{-1}$ ), redox (mV), and C:N. In contrast, neither soil N<sub>2</sub>O production and denitrification potentials were not related to any marsh site-level soil properties ( $r^2=0.20$ ,  $p=0.20$  and  $r^2=-0.36$ ,  $p=0.99$ , respectively).

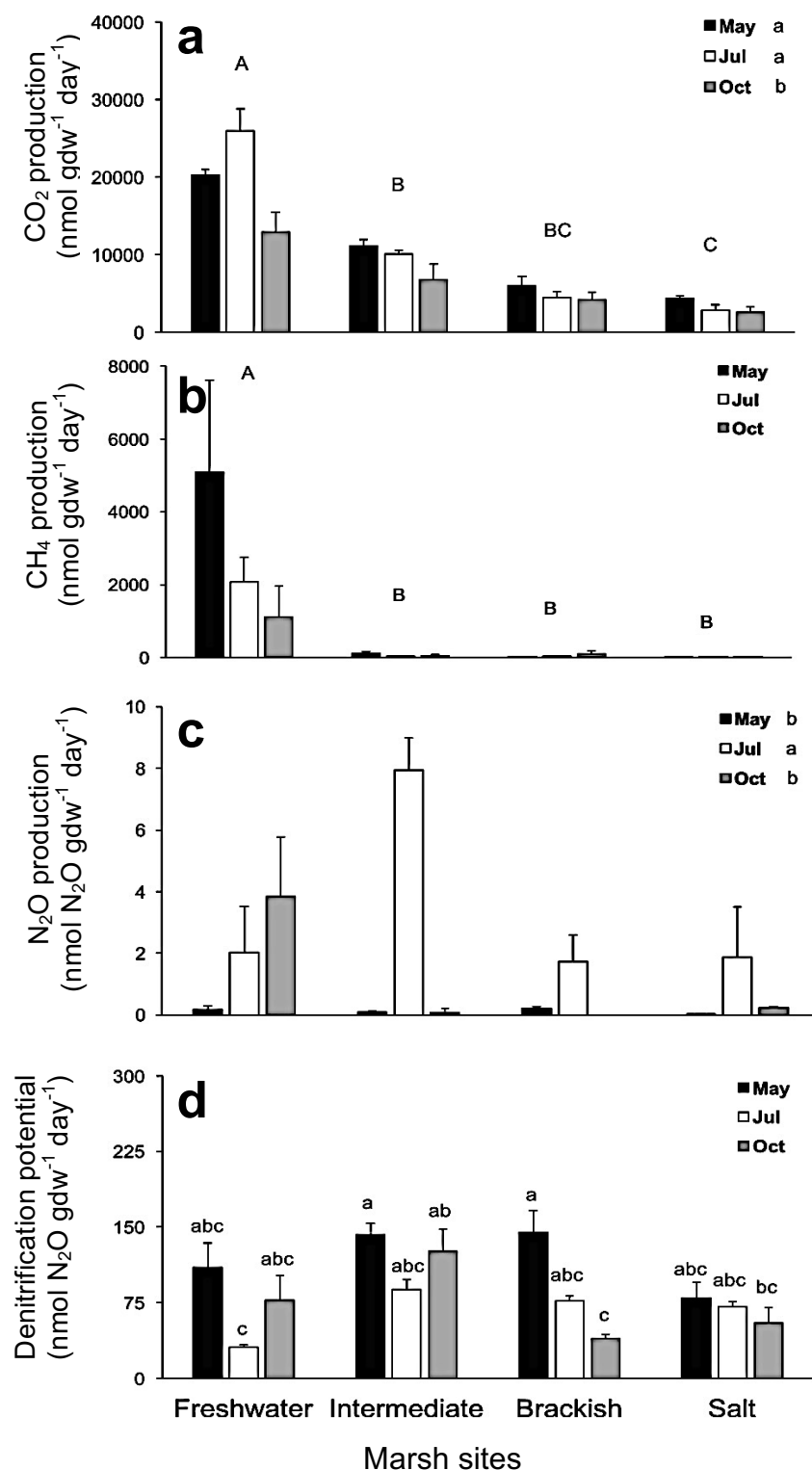


Figure 2. Mean (+standard error) (a) CO<sub>2</sub>, (b) CH<sub>4</sub>, (c) N<sub>2</sub>O production, and (d) denitrification potential along the ambient salinity gradient. Different capital letters represent significant differences between marsh sites. Different lowercase letters next to legend represent significant differences between month. Different lowercase letters above bars represent significant differences between month and marsh site. Significant differences were determined using Tukey's HSD test at  $\alpha=0.05$ .

### **3.3. Effects of salinity alterations on greenhouse gas production and denitrification potential**

During salinity alterations, the CO<sub>2</sub> production increased with salinity from 0 to 30 psu in May (ANOVA;  $p < 0.001$ ; Figure 3; Appendix A, Table A-4) and October ( $p < 0.001$ ). The increases in CO<sub>2</sub> production in response to a short-term exposure of salinity is opposite of the trend along the ambient salinity gradient. Pairwise comparisons were tested when slopes were significantly different (i.e. May and October) and the larger slopes correspond to stronger responses to salinity alterations (Appendix A, Table A-5). Response to salinity (slopes) was significantly different between marsh sites in May ( $p = 0.014$ ) and October ( $p = 0.002$ ), which indicated that CO<sub>2</sub> production in response to salinity decreased from the freshwater to saline marsh soils, which may be related to decline in organic carbon (OC) availability from the freshwater ( $34 \pm 1.2\%$ ), intermediate ( $18 \pm 1.3\%$ ), brackish ( $18 \pm 1.6\%$ ) to saline ( $5.4 \pm 1.2\%$ ) marsh soils. In July, there were no clear patterns of CO<sub>2</sub> production in response to salinity ( $p = 0.71$ ; Figure 3), and differences in slopes between marsh sites were not significant ( $p = 0.56$ ).

The patterns of CO<sub>2</sub> production in response to salinity alterations are strongly correlated with the greater availability of OC ( $r^2 = 0.87$ ,  $p < 0.001$ ; Figure 4) and soils with higher OC (e.g. freshwater marsh) have a stronger response to short-term salinity exposure. Because the July data did not conform to the observed trends for May and October, the regression analysis excluded CO<sub>2</sub> data in July. The absence of response to salinity for July CO<sub>2</sub> production may be attributed to high C:N values. In July, the C:N values in freshwater ( $23 \pm 3.4$ ), intermediate ( $18.5 \pm 2.4$ ), and brackish ( $22 \pm 2.3$ ) were significantly higher than in May and October where C:N ranged from 16 to 18. The

higher C:N values, an indication of lower quality of organic matter, constrained soil respiration with salinity exposure in July.

The increases in salinity had an inhibitory effect on CH<sub>4</sub> production in all months (Figure 3; Appendix A, Table A-4). This response to short-term salinity increases was consistent with the pattern observed along the ambient salinity gradient. When exposed to even the lowest saline amended treatment (5 psu in freshwater and 10 psu in intermediate, brackish and saline marshes), the CH<sub>4</sub> production decreased significantly. When exposed to the 0 psu salinity treatment, the freshwater, intermediate and brackish marshes displayed the highest CH<sub>4</sub> production. Like CO<sub>2</sub> production, the CH<sub>4</sub> production displayed more sensitivity to short-term exposure of salinity in marsh sites with lower salinities where the slopes were highest in the intermediate and freshwater marsh in May (ANOVA;  $p=0.004$ ; Appendix A, Table A-5), however, there were no differences between marsh sites in July ( $p=0.306$ ) or October ( $p=0.344$ ).

In all months, the N<sub>2</sub>O production increased with short-term exposure of salinity and was significantly different between marsh sites (ANOVA; May, July, October:  $p<0.001$ ; Figure 3; Appendix A, Table A-4). The N<sub>2</sub>O production was higher in July than May and October. Pairwise comparisons indicated that the freshwater marsh had the greatest response to salinity in all months (Figure 3; Appendix A, Table A-5).

Greenhouse gases (CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O) respond differently to salinity alterations along the salinity gradient, therefore, the relative radiative forcing that each gas contributes varies (Appendix A, Figure A-2). Methane contributed the greatest relative radiative forcing in the ambient treatment of the freshwater marsh (55%), but decreased to 2% by exposing to 30 psu treatment. Unlike CH<sub>4</sub>, the relative contribution of N<sub>2</sub>O to

the overall radiative forcing increased from 1% in the ambient treatment to 59% in the 30psu treatment. These patterns in CH<sub>4</sub> and N<sub>2</sub>O production were also consistent in the intermediate and brackish marshes when salinities were altered. The saline marsh had low CH<sub>4</sub> and N<sub>2</sub>O production, and the relative radiative forcing was dominated by CO<sub>2</sub> (>86%) for all salinity alterations.

Increasing salinity in short-term exposures resulted in minor declines of DEA rates across most marsh sites (ANOVAs;  $p \leq 0.001$ ; Figure 3; Appendix A, Table A-4). However, slopes were not significantly different across marsh sites, showing that DEA responses were not different ( $p = 0.505, 0.164, 0.320$  in May, July, and October, respectively; Appendix A, Table A-5).

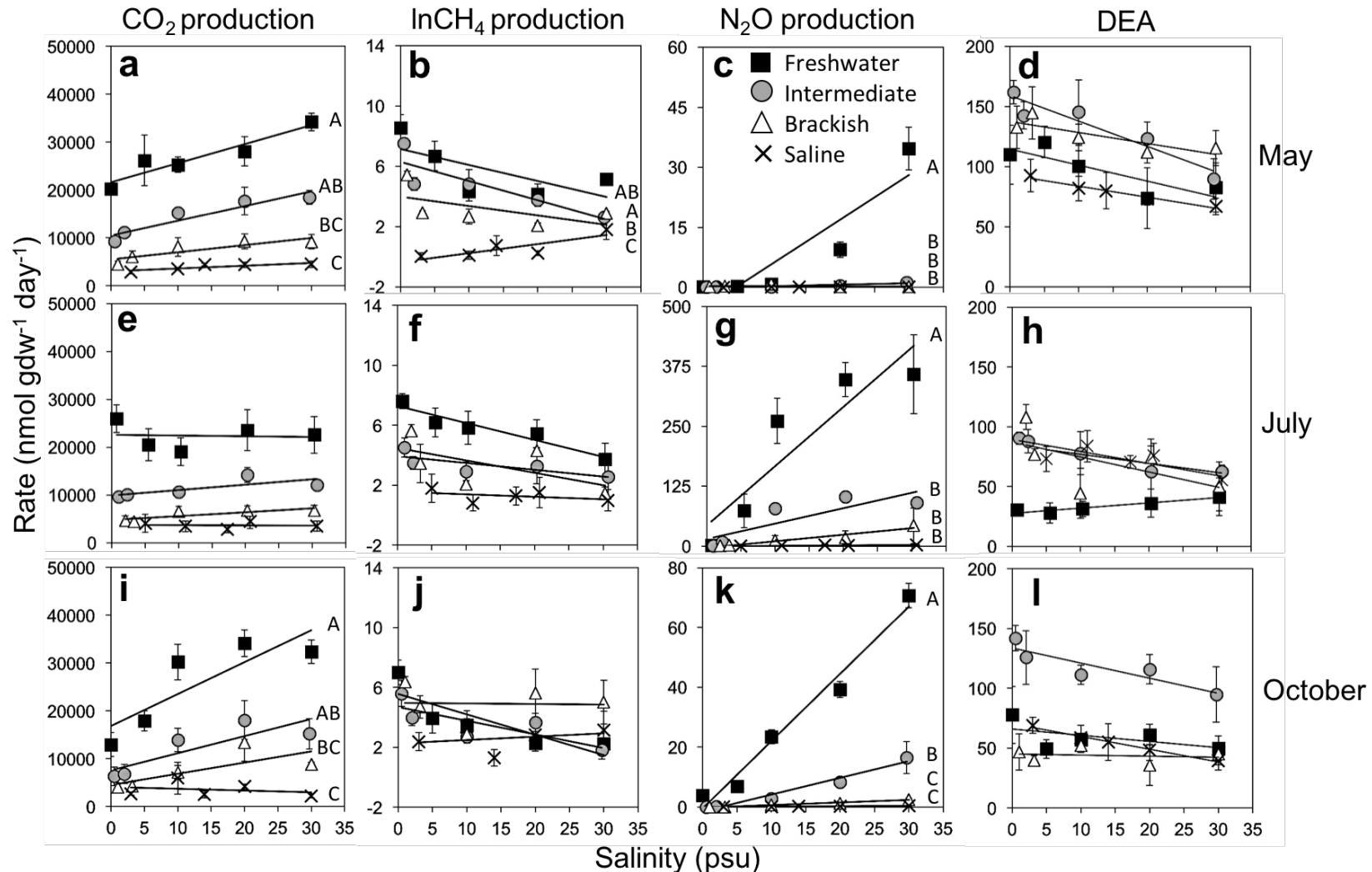


Figure 3. Mean (± standard error) of CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>O production and DEA in response to salinity treatments (n=5) at each marsh site (n=4) in May (a, b, c, d), July (e, f, g, h), and October (i, j, k, l) (n=3). Regression lines indicate responses to salinity at each marsh site within each month. Different capital letters represent significant differences between slopes within each month by pairwise comparisons at  $\alpha=0.05$ . Refer to Appendix A, Table A-5 for individual regressions with equations,  $r^2$ , and p-values.

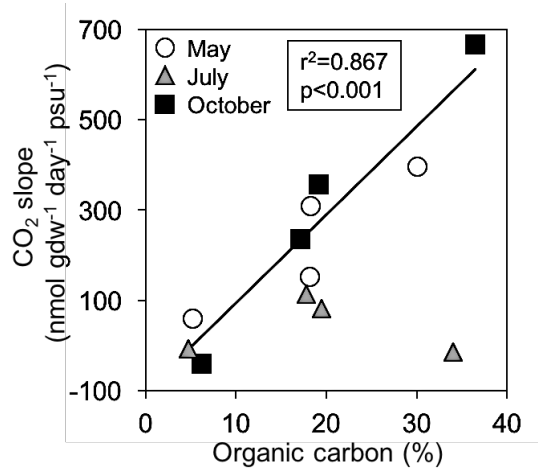


Figure 4. Slope of CO<sub>2</sub> (nmol gdw<sup>-1</sup> day<sup>-1</sup> psu<sup>-1</sup>) in response to organic carbon (%) availability for all marsh sites. A linear regression line in the graph excludes the July data points. Linear regression including July data:  $r^2=0.319$ ,  $p=0.033$ . Refer to section 3.3. for explanations of the July data non-conformity.

## **4. DISCUSSION**

Salinity is a major regulator of C and N cycling in wetland soils. However, the effects of long- and short-term exposure of salinity on soil greenhouse gas (GHG) production and denitrification rates at different marsh sites is not clear. Prior research exists on the effects of salinity exposure on freshwater marshes (e.g. Weston et al. 2006; Weston et al. 2011; Marton et al. 2012), however, much less is known about salinity alterations in brackish and saline marshes. It is critical to understand how environmental conditions that alter salinity (e.g. saltwater intrusion, river diversions) will influence soil C and N losses to the atmosphere at various marsh sites, which have implications for greenhouse gas budgets and nutrient buffering capacity of marsh soils. Major findings from this study were that with long-term exposure to salinity, which shifts vegetation, microbial, and soil properties, may decrease soil CO<sub>2</sub> and CH<sub>4</sub> production. Under short-term exposure to salinity, soil CO<sub>2</sub> production increased (but increases were constrained by quality of organic matter), as well as N<sub>2</sub>O production, however, CH<sub>4</sub> production decreased. The GHG production had the greatest response to short-term salinity exposure in freshwater marsh and declined to the saline marsh.

### **4.1. Soil biogeochemical processes along the ambient salinity gradient**

Salinity is an important variable in determining plant assemblages (Odum et al. 1988). Existing salinity gradients in coastal Louisiana have been classified by differences in vegetation composition (e.g. freshwater, intermediate, brackish, saline; Sasser et al. 2014), which can influence ecosystem functioning (i.e. soil biogeochemical processes). Under anoxic conditions, the accumulation of H<sub>2</sub>S in freshwater marshes may be toxic to freshwater plants, leading to growth inhibition (Koch et al. 1990). In this

study, unvegetated soil slurry incubations were used to determine biogeochemical process rates, however, it is acknowledged that wetland plants influence C and N cycling under field conditions. For example, plants may be an important driver in anaerobic microbial processes that control the turnover of organic C in wetland soils, which may change along the ambient salinity gradient.

The soil CO<sub>2</sub> and CH<sub>4</sub> production rates displayed relationships with several soils properties, whereas the N<sub>2</sub>O production and denitrification potential rates did not. The organic matter, organic C, total N, total P, water content, redox, C:N, and extractable PO<sub>4</sub><sup>3-</sup> declined from the freshwater to saline marsh, whereas, porewater salinity, bulk density and pH increased. Long-term exposure to salinity may result in an increase in total organic C mineralization due to a higher rate of SO<sub>4</sub><sup>2-</sup> reduction (Weston et al. 2006; Weston et al. 2011).

In the GHG experiment, soil CO<sub>2</sub> and CH<sub>4</sub> production decreased with salinity, which is consistent with several studies (Smith et al. 1983; Nyman and DeLaune 1991; Poffenbarger et al. 2011; Holm et al. 2016). Regressions of PCA dimensions (1 and 2) with ambient CO<sub>2</sub> and CH<sub>4</sub> production revealed a positive correlation between these rates and several marsh site-level soil properties, showing that differences in soil properties may be a driver in C losses to the atmosphere. This indicates that a long-term exposure to salinity (e.g. saltwater intrusion) in wetland soils may reduce organic C pools and increase organic matter recalcitrance (Neubauer et al. 2013; Weston et al. 2011), supporting reduced soil CO<sub>2</sub> production in the saline marsh. In terms of relative radiative forcing, CH<sub>4</sub> was the greatest contributor in the freshwater marsh (Appendix A, Figure A-1). Temporal patterns over the course of the growing season revealed higher

CO<sub>2</sub> production rates in May and July compared to October, consistent with greater soil respiration rates with temperature (Davidson et al. 1998).

Soil CH<sub>4</sub> production rates declined > 97% from the freshwater to intermediate marsh despite mean porewater salinity only increasing from 0.11 to 1.5psu. Methane production decreased 25% from the intermediate to brackish marsh (1.5 to 2.7 psu, respectively). Methanogenesis is a dominant organic C mineralization microbial pathways in freshwater marshes, whereas methanogenesis in brackish marshes is replaced by iron (Fe(III)) and SO<sub>4</sub><sup>2-</sup> reduction (Neubauer et al. 2005). In October, the porewater H<sub>2</sub>S in the freshwater and intermediate marshes (0.15±0.07 µg gdw<sup>-1</sup>) was lower than brackish and saline marshes (2.9±1.1 µg gdw<sup>-1</sup>; p=0.002), consistent with increased SO<sub>4</sub><sup>2-</sup> reduction at higher salinities (Weston et al. 2006; Weston et al. 2011).

In contrast to soil CO<sub>2</sub> and CH<sub>4</sub> production at ambient salinity, the N<sub>2</sub>O production rates in the GHG experiments were low (<10 N<sub>2</sub>O nmol gdw<sup>-1</sup> day<sup>-1</sup>), did not vary across marsh sites, and were not correlated to marsh site-level soil properties. N<sub>2</sub>O emissions can occur through two microbial pathways: denitrification (Knowles 1982) and nitrification (Yoshida and Alexander 1970). In the GHG laboratory controlled experiments, flasks were anoxic, thereby inhibiting nitrification. High denitrification observed in other studies were positively correlated with NO<sub>3</sub><sup>-</sup> availability (Nielsen et al. 1995; Moseman-Valtierra et al. 2011). If denitrification rates are more limited by N than C, then it is possible that N<sub>2</sub>O production spatial patterns were not observed in this experiment because extractable NO<sub>3</sub><sup>-</sup> concentrations did not vary across marsh sites (ANOVA; p=0.603). Even though experiments were conducted in a laboratory controlled environment (approximately 20 to 25°C), mean N<sub>2</sub>O production rates at ambient salinity

were significantly higher in July (peak summer) than May and October. High temperatures could have impacted microbial communities in the soil cores collected in July. Denitrification rates may increase with temperature (Nowicki 1994).

The denitrification potential varied between marsh sites and month, whereas the rates tended to be highest in May in the intermediate ( $142 \pm 11 \text{ nmol gdw}^{-1} \text{ d}^{-1}$ ) and brackish ( $145 \pm 21 \text{ nmol gdw}^{-1} \text{ d}^{-1}$ ) marsh. High denitrification rates may be correlated with freshwater marsh soils (higher C and N content), whereas low rates are correlated with saline marsh soils because salinity is inversely correlated with C and N content (Craft 2007; Dodla et al. 2008). The lack of observed spatial patterns in soil extractable  $\text{NO}_3^-$  concentrations could have led to variable denitrification rates upon initial core collection, which influenced subsequent results from the laboratory controlled experiments. Increased salinity can decrease denitrification rates driven by  $\text{H}_2\text{S}$  toxicity (Giblin et al. 2010; Osborne et al. 2015).

#### **4.2. Effects of salinity pulses on soil biogeochemistry**

Soil  $\text{CO}_2$  production rates generally increased with short-term exposure to salinity from 0 to 30psu in May and October, and the magnitude of increase was highest in the freshwater marsh, declining to the saline marsh. Chambers et al. (2011) and Weston et al. (2011) found similar trends of increased  $\text{CO}_2$  flux rates (20-32% and 21%, respectively), when freshwater wetland soils were exposed short-term salinity increases. The effects of short-term salinity (0 to 30 psu) on  $\text{CO}_2$  production rates were most pronounced in the freshwater marsh in May (69% increase) and October (150% increase), likely attributed to greater organic C availability and  $\text{SO}_4^{2-}$  inputs serving as terminal electron acceptors during anaerobic microbial respiration, leading to high rates

of gaseous C (in the form of CO<sub>2</sub>) loss. Saline marsh soils were least responsive to salinity alterations in all months, may be due to interstitial presence of salt and SO<sub>4</sub><sup>2-</sup> in cores upon collection, where ambient porewater salinities ranged from 14 to 18 psu. These results indicate that despite high soil CO<sub>2</sub> production in a freshwater marsh, an exposure to 0 psu in a saline marsh does not necessarily increase soil respiration rates.

A positive relationship between soil CO<sub>2</sub> production in response to salinity and soil organic C in May and October (Figure 4;  $p < 0.001$ ;  $r^2 = 0.867$ ) was observed. Interestingly, July did not conform to this relationship, which may be attributed to the lower quality of organic matter shown by high C:N values in the freshwater ( $23 \pm 3.4$ ), intermediate ( $18.5 \pm 2.4$ ), and brackish ( $22 \pm 2.3$ ) marshes, compared to May and October, which ranged from 16 to 18. High C:N ratios in July may indicate a low labile C availability and more recalcitrant organic matter (Neubauer et al. 2013), leading to a reduction soil CO<sub>2</sub> emissions during the salinity alteration experiments.

Contrary to high rates of soil CO<sub>2</sub> production, soil CH<sub>4</sub> production drastically from the 0 psu to the next lowest salinity treatment (ambient porewater or 5 psu) in the freshwater and intermediate marsh, suggesting that CH<sub>4</sub> is largely inhibited by even low-salinity saltwater. In Marton et al. (2012), salinity (even at low levels; e.g. 2 psu) drastically decreased CH<sub>4</sub> production in the freshwater soils. The CH<sub>4</sub> production rate was highest in freshwater marsh soil, consistent with prior research demonstrating methanogenesis to be the dominant pathway (62%) of organic matter mineralization in freshwater soils (Weston et al. 2006). Decline in CH<sub>4</sub> production in response to short-term salinity exposure was consistent with the decline in CH<sub>4</sub> production along the ambient salinity gradient from the freshwater to saline marsh. Short-term pulses of

freshwater moderately increased  $\text{CH}_4$  production rates in the intermediate and brackish marshes. Exposing freshwater into the intermediate and brackish marshes may decrease  $\text{SO}_4^{2-}$  and  $\text{H}_2\text{S}$  availability, increasing methanogenesis.

Linear relationships between salinity and soil  $\text{N}_2\text{O}$  production were revealed in most marsh sites, with the highest rates measured in July. Short-term exposure to salinity and accumulation of  $\text{H}_2\text{S}$  likely inhibited the reduction of  $\text{N}_2\text{O}$  to  $\text{N}_2$  by serving as an alternate electron donor (Sorensen 1978) and inhibiting both nitrification and denitrification (Joye and Hollibaugh 1995). There were particularly high rates of  $\text{N}_2\text{O}$  production in the freshwater marsh soil exposed to short-term salinity increases. Freshwater marsh site-level soil properties (organic matter, C, and N content) were about 2 times greater than in the intermediate/brackish marshes, and 4 to 6 times greater than in the saline marsh. High organic matter, C and N availability favors conditions for higher denitrification rates (Dodla et al. 2008). Furthermore, the freshwater marsh site-level pH was low ( $5.7 \pm 0.1$ ) compared to brackish ( $7.0 \pm 0.2$ ) and saline ( $7.2 \pm 0.4$ ) marsh, which is a favorable condition for incomplete denitrification (Knowles 1982) which prevents the reduction of  $\text{N}_2\text{O}$  to  $\text{N}_2$  in the flasks by inhibition of the enzyme nitrous oxide reductase (Seitzinger 1988).

The denitrification potential exhibited minor decreases with salinity pulses, although this was not significant. Higher salinities may have increased  $\text{SO}_4^{2-}$  reduction (leading to higher  $\text{CO}_2$  emissions) and replaced denitrification as the dominant pathway (Weston et al. 2006) for microbial respiration due to the presence of alternate electron donor ( $\text{H}_2\text{S}$ ). Increases in salinity may reduce the N removal capacities of marsh soils through the pathway of denitrification.

### 4.3. Conclusions

Collectively, these findings indicated that higher salinity marsh soils, particularly saline marshes, displayed the weakest GHG production and denitrification responses to short-term freshwater and saltwater inputs, whereas some rates in freshwater marshes (i.e. CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O production) were highly responsive. Previous research has predominantly focused on the biogeochemical responses of saltwater intrusion into freshwater soils (Chambers et al. 2011; Weston et al. 2011; Marton et al. 2012; Neubauer et al. 2013), but less information is available on the effects of freshwater and saltwater inputs in intermediate, brackish, and saline marshes. Soil properties, such as organic matter quantity and quality control GHG production and denitrification rates in both long- and short-term exposures of salinity.

The proposed sediment diversion in mid-Barataria Basin in southeastern Louisiana will introduce fresh and nutrient rich (e.g. N, P, Si, trace elements) Mississippi River water into the intermediate, brackish, and saline marsh sites. River diversions may also inundate wetlands and decrease O<sub>2</sub> availability, resulting in changes of biogeochemical process rates. Results in the study indicate an interactive effect of salinity on GHG production and denitrification rates. Restoration activities that take this effect into account may help reduce GHG release into the atmosphere and increase the N buffering capacities of coastal wetlands. For example, inputs of freshwater into receiving wetlands from river diversions may lead to sustained shifts to fresher conditions (altering vegetation composition, microbial communities, and soil properties closer to freshwater marsh conditions), which may increase the overall CO<sub>2</sub> and CH<sub>4</sub> production in wetlands. As a result, the location of these river diversions may be best

suited to an area closest to 0 psu to limit the presence of freshwater in intermediate and brackish marshes. Although short-term releases of freshwater increases  $\text{CH}_4$  production, it may simultaneously decrease  $\text{CO}_2$  and  $\text{N}_2\text{O}$  production, leading to tradeoffs between certain GHG releases and removal of N. Greenhouse gas production and denitrification rates may be greatest when nutrient availability and temperatures are high, therefore diverting water during winter months may maintain current biogeochemical process rate trends along the salinity gradient.

Globally, sea level rise and other anthropogenic modifications (e.g. dredging canals) are altering coastal landscapes, making this study applicable to other estuarine systems. This study shows that the inputs of fresh and saltwater in short- and long-term timescales may affect marsh soil C and N losses differently, which may require a re-evaluation of the C and N balance of soils, especially when considering the vulnerability of coastal wetlands to changes in salinity.

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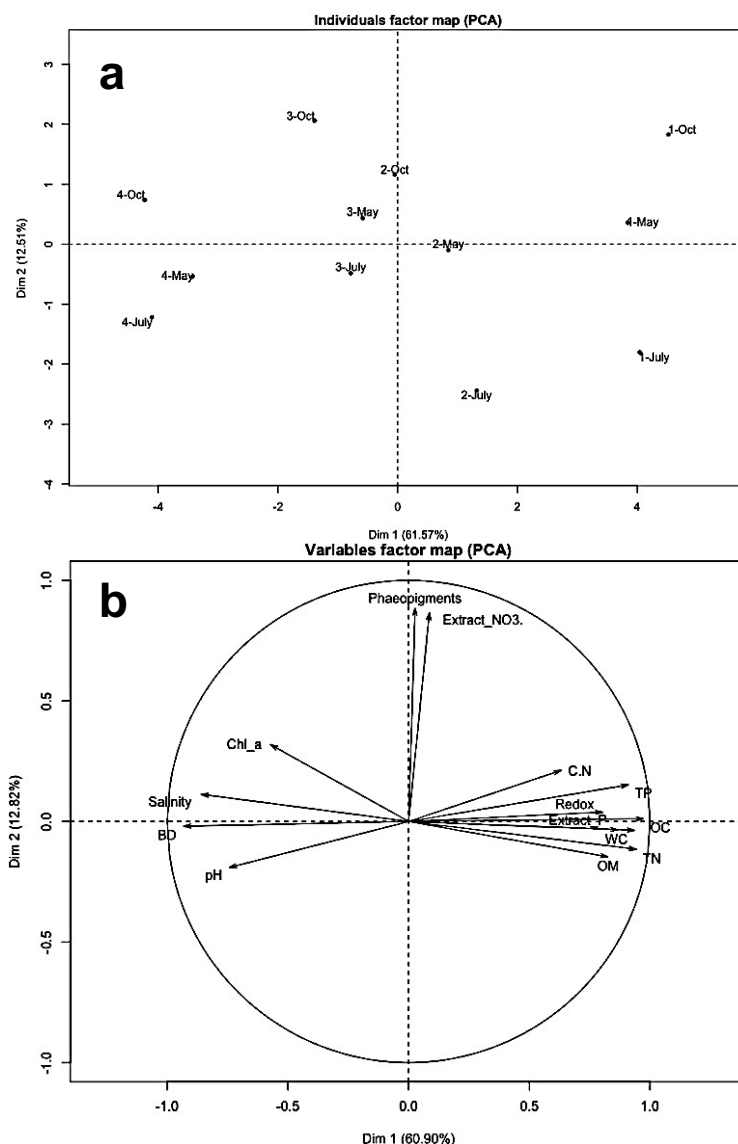
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## APPENDIX A: SUPPLEMENTAL MATERIALS



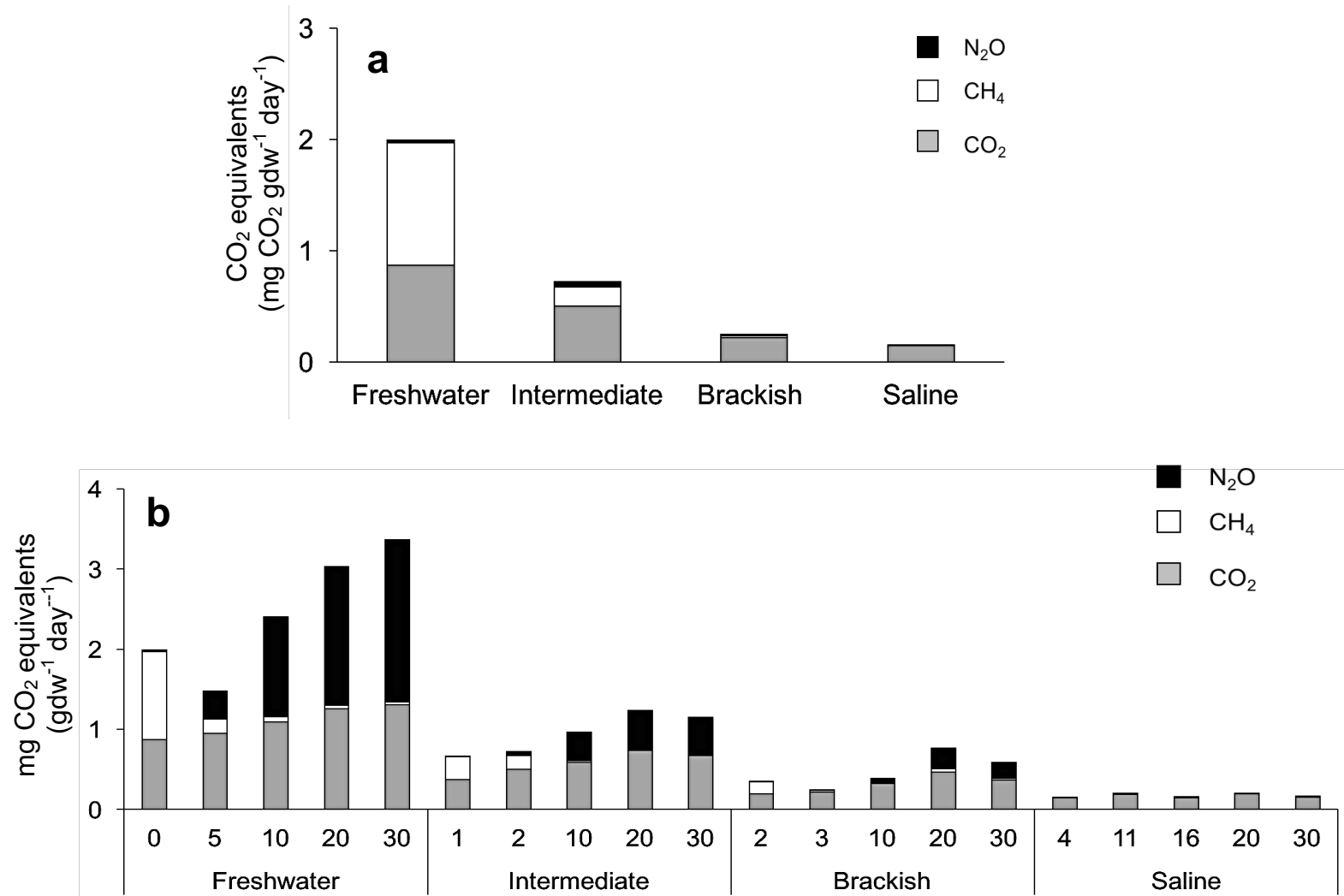
Supplementary Figure A-1. (a) The results of a PCA of Individual factors (marsh site and month,  $n=12$ ) map of PCA where 1=freshwater, 2=intermediate, 3=brackish, 4=saline. Dimension 1 explains 61% of the variation and dimension 2 explains 13% of the variation and (b) PCA of soil variables as vectors ( $n=14$ ). Dimension 1 explains 61% of the variation and dimension 2 explains 13% of the variation. The soil variables on positive dimension 1: organic matter (OM), total nitrogen (TN), organic carbon (OC), extractable phosphate (Extractable\_P), total phosphorus (TP), redox, C:N (C.N). The soil variables on negative dimension 1: bulk density (BD), porewater salinity (Salinity), pH, chlorophyll a (Chl\_a). The soil properties on dimension 2: Phaeopigments and extractable  $\text{NO}_3^-$  (Extract\_NO3-). Perpendicular vectors are uncorrelated. Vectors with small angles between them are positively correlated to each other. Opposite vectors are negatively correlated. The longer lengths of vectors indicate variability.

Supplementary Table A-1. Summary of two-way ANOVA results displaying CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>O production and DEA at ambient salinities with marsh site (n=4) and month (n=3) as main effects.

<b>Response</b>	<b>Effect</b>	<b>Df</b>	<b>F</b>	<b>p</b>
CO <sub>2</sub>	Month	2	7.0	0.004*
	Marsh site	3	48.8	<0.001*
	Month~Marsh site	6	0.9	0.50
CH <sub>4</sub>	Month	2	0.7	0.50
	Marsh site	3	46.3	<0.001*
	Month~Marsh site	6	1.6	0.20
N <sub>2</sub> O	Month	2	16.9	<0.001*
	Marsh site	3	3.6	0.03*
	Month~Marsh site	6	1.8	0.16
DEA	Month	2	15.8	<0.001*
	Marsh site	3	8.3	<0.001*
	Month~Marsh site	6	5.4	0.002*

~Denotes interaction

\*Denotes significance at  $\alpha=0.05$



Supplementary Figure A-2. (a) Soil radiative forcing in CO<sub>2</sub> equivalents for CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O along the ambient salinity gradient averaged across all months and (b) soil radiative forcing in CO<sub>2</sub> equivalents for CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O along the ambient salinity gradient in response to salinity alterations (psu) averaged across all months.

Supplementary Table A-2. Summary of linear models of CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>O production and DEA in response to soil properties in dimensions 1 and 2. Significant positive estimates indicate positive relationship with dimension soil properties and negative estimates indicate correlation with negative dimension soil properties. Refer to Figure A-1.

Response	Variables	Estimate	Standard error	t value	p
CO <sub>2</sub>	<sup>1</sup> Dim. 1	2040	369	5.5	<0.001*
	<sup>2</sup> Dim. 2	1406	804	1.7	0.114
CH <sub>4</sub>	Dim. 1	314	126	2.5	0.034*
	Dim. 2	136	275	0.49	0.633
N <sub>2</sub> O	Dim. 1	0.25	0.24	1.04	0.325
	Dim. 2	0.23	0.52	0.43	0.678
DEA	Dim. 1	1.2	3.6	0.33	0.748
	Dim. 2	-12	7.9	-1.5	0.173

<sup>1</sup>Positive dimension 1 soil properties: organic matter, total nitrogen, organic carbon, C:N, extractable phosphate, total phosphorus, and redox. <sup>1</sup>Negative dimension 1 soil properties: bulk density, porewater salinity, pH, and chlorophyll a

<sup>2</sup>Dimension 2 soil properties: phaeopigments and extractable nitrate

Supplementary Table A-3. ANCOVA models with marsh site (n=4) and salinity (continuous covariate) as independent variables in May, July and October for each response (CO<sub>2</sub>, lnCH<sub>4</sub>, N<sub>2</sub>O and DEA). Adjusted R<sup>2</sup> indicates the amount of variability explained by the independent variables.

Response	Month	Residual SE	df	Multiple R <sup>2</sup>	Adjusted R <sup>2</sup>	F	p
CO <sub>2</sub>	May	3009	52	0.915	0.938	80.2	<0.001*
	July	3342	55	0.834	0.825	93.8	<0.001*
	October	4743	52	0.817	0.793	33.21	<0.001*
lnCH <sub>4</sub>	May	1.25	52	0.763	0.731	23.9	<0.001*
	July	1.52	55	0.586	0.556	19.5	<0.001*
	October	1.53	55	0.349	0.301	7.36	<0.001*
N <sub>2</sub> O	May	3.564	52	0.822	0.798	34.37	<0.001*
	July	53.48	52	0.822	0.798	34.28	<0.001*
	October	3.456	52	0.966	0.961	211.2	<0.001*
DEA	May	25.7	55	0.526	0.492	15.3	<0.001*
	July	22.8	55	0.419	0.376	9.90	<0.001*
	October	21.0	55	0.688	0.665	30.3	<0.001*

\*Denotes significance at  $\alpha=0.05$

Supplementary Table A-4. Two-way ANOVA type III results for each month with main effects as marsh site (n=4) and salinity for CO<sub>2</sub>, lnCH<sub>4</sub>, N<sub>2</sub>O and DEA responses.

Month	Effect	Sum sq	Df	F	p
<b>CO<sub>2</sub></b>					
May	(Intercept)	2861487587	1	316	<0.001*
	Marsh site	1136323255	3	41.8	<0.001*
	Salinity	272404271	1	30.1	<0.001*
	Marsh site~Salinity	105229055	3	3.87	0.014*
July	(Intercept)	3016707319	1	266	<0.001*
	Marsh site	1206881934	3	35.5	<0.001
	Salinity	1584984	1	0.140	0.710
	Marsh site~Salinity	23425179	3	0.690	0.563
October	(Intercept)	1765041174	1	78.5	<0.001*
	Marsh site	588513243	3	8.72	<0.001*
	Salinity	761215409	1	33.8	<0.001*
	Marsh site~Salinity	372313157	3	5.52	0.002*
<b>lnCH<sub>4</sub></b>					
May	(Intercept)	255	1	163	<0.001*
	Marsh site	133	3	28.5	<0.001*
	Salinity	13.8	1	8.83	0.004*
	Marsh site~Salinity	23.5	3	5.02	0.004*
July	(Intercept)	266	1	117	<0.001*
	Marsh site	71.5	3	10.4	<0.001*
	Salinity	27.4	1	12.0	0.001*
	Marsh site~Salinity	8.46	3	1.24	0.306
October	(Intercept)	142	1	60.9	<0.001*
	Marsh site	19.2	3	2.75	0.052
	Salinity	21.5	1	9.22	0.004*
	Marsh site~Salinity	7.92	3	1.13	0.344
<b>N<sub>2</sub>O</b>					
May	(Intercept)	180.53	1	14.2	<0.001*
	Marsh site	138.33	3	3.63	0.019*
	Salinity	2172.57	1	171	<0.001*
	Marsh site~Salinity	1564.62	3	41.0	<0.001*
July	(Intercept)	11121	1	3.89	0.054
	Marsh site	7463	3	0.870	0.463
	Salinity	256443	1	89.7	<0.001*
	Marsh site~Salinity	145205	3	16.9	<0.001*
October	(Intercept)	0.6	1	0.053	0.818
	Marsh site	3.8	3	0.107	0.956
	Salinity	8832	1	740	<0.001*
	Marsh site~Salinity	5366	3	150	<0.001*
<b>DEA</b>					
May	(Intercept)	80146	1	120	<0.001*
	Marsh site	12612	3	6.28	0.001*

July	Salinity	3084	1	4.61	0.037*
	Marsh site~Salinity	1586	3	0.790	0.505
	(Intercept)	4648	1	9.31	0.004*
	Marsh site	14858	3	9.92	<0.001*
	Salinity	258	1	0.517	0.476
	Marsh site~Salinity	2649	3	1.77	0.164
October	(Intercept)	26427	1	60.5	<0.001*
	Marsh site	28945	3	22.1	<0.001*
	Salinity	455	1	1.04	0.311
	Marsh site~Salinity	1569	3	1.20	0.320

~Denotes interaction

\*Denotes significance at  $\alpha=0.05$

Supplementary Table A-5. Individual linear regressions at each marsh site (n=4) and in each month (n=3) in response to salinity alterations for CO<sub>2</sub>, InCH<sub>4</sub>, N<sub>2</sub>O production and DEA responses.

Month	Marsh site	Regression equation	r <sup>2</sup>	p
<b>CO<sub>2</sub></b>				
May	Freshwater	CO <sub>2</sub> =21649+396*salinity	0.89	<0.001*
	Intermediate	CO <sub>2</sub> =10525+308*salinity	0.89	<0.001*
	Brackish	CO <sub>2</sub> =5454+152*salinity	0.78	0.018*
	Saline	CO <sub>2</sub> =2995+60*salinity	0.74	0.017*
July	Freshwater	CO <sub>2</sub> =22590-15*salinity	0.005	0.834
	Intermediate	CO <sub>2</sub> =9915+112*salinity	0.57	0.024*
	Brackish	CO <sub>2</sub> =4782+81*salinity	0.69	0.074
	Saline	CO <sub>2</sub> =3827-8*salinity	0.01	0.913
October	Freshwater	CO <sub>2</sub> =16850+666*salinity	0.72	<0.001*
	Intermediate	CO <sub>2</sub> =7612+357*salinity	0.71	0.010*
	Brackish	CO <sub>2</sub> =4516+235*salinity	0.55	0.010*
	Saline	CO <sub>2</sub> =4154-41*salinity	0.07	0.484
<b>InCH<sub>4</sub></b>				
May	Freshwater	InCH <sub>4</sub> =7.1-0.11*salinity	0.47	0.049*
	Intermediate	InCH <sub>4</sub> =6.3-0.13*salinity	0.76	<0.001*
	Brackish	InCH <sub>4</sub> =4.0-0.06*salinity	0.33	0.044*
	Saline	InCH <sub>4</sub> =-0.35+0.06*salinity	0.7	0.034*
July	Freshwater	InCH <sub>4</sub> =7.3-0.11*salinity	0.91	0.004
	Intermediate	InCH <sub>4</sub> =3.9-0.05*salinity	0.59	0.023*
	Brackish	InCH <sub>4</sub> =4.5-0.08*salinity	0.35	0.120
	Saline	InCH <sub>4</sub> =1.6-0.02*salinity	0.17	0.647
October	Freshwater	InCH <sub>4</sub> =5.6-0.14*salinity	0.72	0.008*
	Intermediate	InCH <sub>4</sub> =4.7-0.09*salinity	0.63	0.005*
	Brackish	InCH <sub>4</sub> =5.0-0.005*salinity	0.002	0.219
	Saline	InCH <sub>4</sub> =2.2+0.02*salinity	0.08	0.901

<b>N<sub>2</sub>O</b>				
May	Freshwater	$N_2O = -5.4 + 1.1 * \text{salinity}$	0.82	<0.001*
	Intermediate	$N_2O = -0.07 + 0.04 * \text{salinity}$	0.81	<0.001*
	Brackish	$N_2O = 0.35 - 0.004 * \text{salinity}$	0.03	0.762
	Saline	$N_2O = 0.008 + 0.002 * \text{salinity}$	0.79	0.025*
July	Freshwater	$N_2O = 42 + 12 * \text{salinity}$	0.82	<0.001*
	Intermediate	$N_2O = 14 + 3.2 * \text{salinity}$	0.72	<0.001*
	Brackish	$N_2O = -3.5 + 1.4 * \text{salinity}$	0.94	0.073
	Saline	$N_2O = 0.04 + 0.08 * \text{salinity}$	0.87	0.114
October	Freshwater	$N_2O = -0.39 + 2.2 * \text{salinity}$	0.97	<0.001*
	Intermediate	$N_2O = -1.4 + 0.56 * \text{salinity}$	0.96	<0.001*
	Brackish	$N_2O = -0.17 + 0.08 * \text{salinity}$	0.97	<0.001*
	Saline	$N_2O = -0.03 + 0.01 * \text{salinity}$	0.81	<0.001*
<b>DEA</b>				
May	Freshwater	$DEA = 115 - 1.3 * \text{salinity}$	0.70	0.005*
	Intermediate	$DEA = 159 - 2.1 * \text{salinity}$	0.90	0.005*
	Brackish	$DEA = 138 - 0.92 * \text{salinity}$	0.70	0.136
	Saline	$DEA = 93 - 0.92 * \text{salinity}$	0.96	0.070
July	Freshwater	$DEA = 28 + 0.42 * \text{salinity}$	0.89	0.322
	Intermediate	$DEA = 89 - 1.0 * \text{salinity}$	0.91	0.095
	Brackish	$DEA = 87 - 1.3 * \text{salinity}$	0.36	0.111
	Saline	$DEA = 85 - 0.77 * \text{salinity}$	0.54	0.155
October	Freshwater	$DEA = 66 - 0.52 * \text{salinity}$	0.30	0.345
	Intermediate	$DEA = 134 - 1.3 * \text{salinity}$	0.79	0.061
	Brackish	$DEA = 45 - 0.10 * \text{salinity}$	0.03	0.855
	Saline	$DEA = 71 - 1.1 * \text{salinity}$	0.99	0.029*

\*Denotes significance at  $\alpha=0.05$

## APPENDIX B: AUGUST 2015 PILOT STUDY

### B.1. Overview

A pilot study was conducted in August 2015 to gather exploratory data and assess the feasibility of the proposed work (refer to thesis proposal). This appendix describes the pilot study's basic overview, the modifications that were made for the 2016 field season, and the main results.

Five sites were chosen along a salinity gradient in Barataria Bay, LA, USA by their vegetation classification (Sasser et al. 2014). These 5 sites included a freshwater, intermediate, brackish, saline marsh (same sites as thesis study) and mangrove (*Avicennia germinans*) stand. Three replicate soil cores (5.7 cm diameter x 5 cm depth) and vegetation, soil, and water samples were collected in the same experimental plot design as thesis. All samples were put on ice until return to the laboratory. Once at the laboratory, soil properties such as porewater salinity, bulk density, water content, organic matter, organic carbon, organic total nitrogen, total phosphorus, extractable nutrients and pH were measured. Greenhouse gas production and denitrification enzyme activity laboratory experiments were conducted in the same design as thesis experiments, but only three incubated salinities (-5psu from ambient porewater salinity, ambient porewater salinity, +5psu from ambient porewater salinity and 0, 2, 5psu for the freshwater marsh) were used.

Based on the preliminary sampling in August 2015, plant diversity decreased with increasing salinities. The freshwater site had the highest species diversity and was dominated by *Sagittaria platyphylla* (delta arrowhead) and *Panicum hemitomon* (maidencane) and it contained 8 other species. The intermediate site was dominated by

*Polygonum hydropiper* (marshpepper knotweed) and contained 5 other species. The brackish marsh was co-dominated by *Spartina patens* (saltmeadow cordgrass) and *S. alterniflora* (smooth cordgrass), with *Distichlis spicata* (saltgrass) was present. The saline marsh was dominated by *S. alterniflora* and the mangrove stand was dominated by *Avicennia germinans*.

## **B.2. Modifications**

This pilot study provided useful insight on how to best answer our research questions. The modifications from this pilot study to the thesis sampling were as follows:

- Sample from the freshwater, intermediate, brackish and saline marsh (exclude black mangrove (*Avicennia germinans*) stand).
- Include a broader range of salinity alterations (0, 10, 20, 30psu) plus ambient salinity and (5, 10, 20, 30psu) plus ambient salinity for the freshwater marsh.
- Attempt to reach target salinities by measuring porewater salinity and water content of soil prior to experiments and treating with appropriate saline solution.
- Measure salinity after experiments to obtain true salinity measurements to use in regression analyses to correlate salinity and responses.
- Increase amount of soil (15 g to 20 g) in experiments to detect N<sub>2</sub>O production.

## **B.3. Results**

### **B.3.1. Greenhouse gas production and denitrification potential along the ambient salinity gradient**

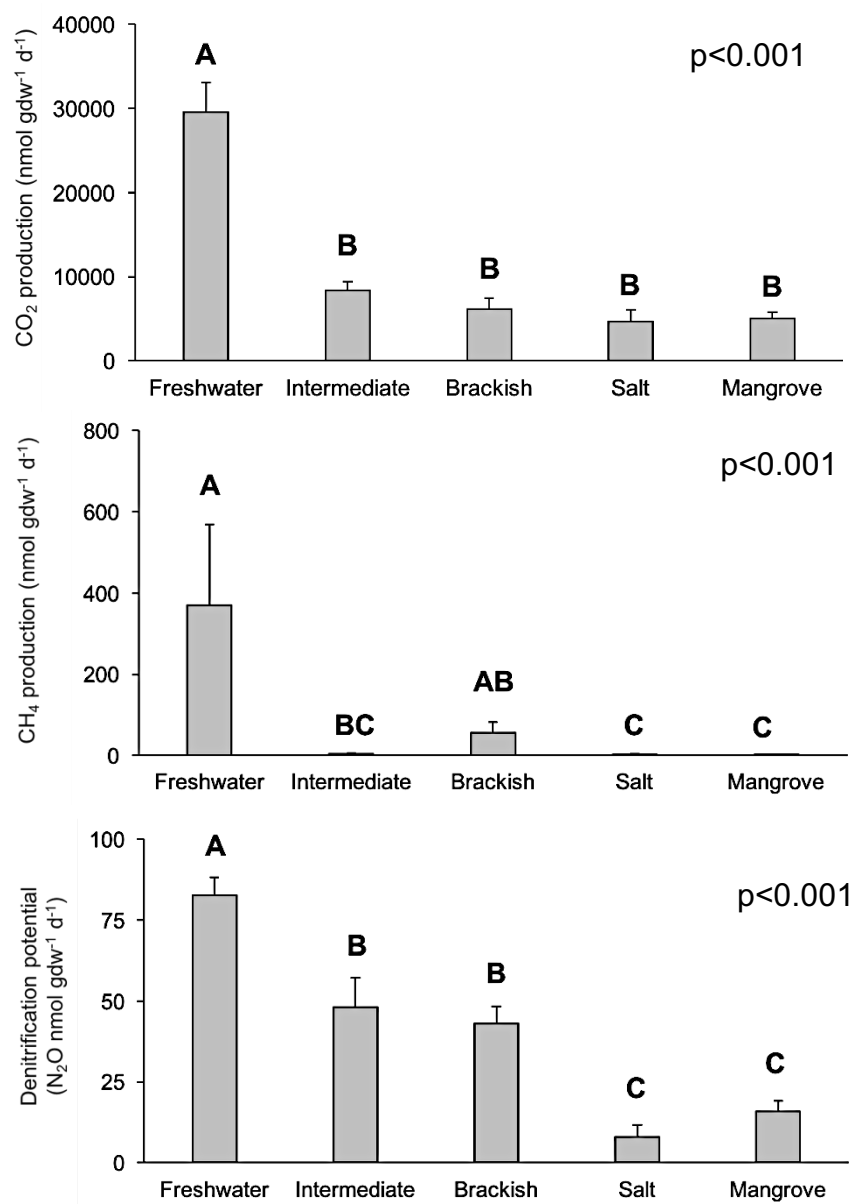
Similar to results from the main study, the pilot study exhibited a decline in CO<sub>2</sub> production (ANOVA;  $p < 0.001$ ) and CH<sub>4</sub> production ( $p < 0.001$ ) from the freshwater to saline marsh and mangrove. However, in the pilot study, denitrification potentials decreased from the freshwater to saline marsh and mangrove ( $p < 0.001$ ; Figure 5).

### **B.3.2. Soil properties**

From the freshwater to saline marsh and mangrove, porewater salinity, pH, and bulk density tended to increase (Table 5). The organic matter availability, organic carbon, total nitrogen and extractable nitrate ( $\text{NO}_3^-$ ) was higher in the freshwater marsh than in the intermediate, brackish, saline marsh and mangrove sites (Table 6), which may have contributed to these higher  $\text{CO}_2$  and  $\text{CH}_4$  production and denitrification potentials in the freshwater marsh.  $\text{CO}_2$  and  $\text{CH}_4$  production and denitrification potential rates at ambient salinities were significantly correlated with several soil properties (Table 7, 8, and 9).

### **B.3.3. Effects of salinity alterations on greenhouse gas production and denitrification potential**

Overall, soil  $\text{CO}_2$  production and denitrification potential responses to salinity were not significant, whereas  $\text{CH}_4$  production declined. Similar to results from the main study,  $\text{CH}_4$  production decreased with elevated salinity treatments (ANOVA;  $p=0.037$ ), whereas  $\text{CO}_2$  ( $p=0.80$ ) and denitrification potential ( $p=0.59$ ) did not change with salinity alterations (Figure 6).  $\text{CO}_2$  production (ANOVA;  $p=0.91$ ),  $\text{CH}_4$  production (ANOVA;  $p=0.98$ ), and denitrification potential (ANOVA;  $p=0.52$ ) were not different between marsh sites in response to salinity alterations.



Pilot Study Figure B-1. Mean (+standard error) of CO<sub>2</sub> production, CH<sub>4</sub> production, and denitrification potential at five different marsh sites (freshwater, intermediate, brackish, salt and mangrove) in August 2015. Different capital letters represent differences between marsh sites. Significant differences were determined using Tukey's HSD test at  $\alpha=0.05$ .

Pilot Study Table B-1. Mean ( $\pm$ standard error) of soil properties at 0 to 5 cm depth along the salinity gradient at different marsh sites (freshwater, intermediate, brackish, salt and mangrove) for the pilot study in August 2015. Different lowercase letters represent significant differences between site means using Tukey's HSD test at  $\alpha=0.05$ .

Marsh site	Porewater salinity (psu)	pH	Redox (mV)	Bulk density (g/cm <sup>3</sup> )	Water content (%)
Freshwater	0c	5.23 $\pm$ 0.11c	144a	0.10 $\pm$ 0.01b	90 $\pm$ 1.54a
Intermediate	4 $\pm$ 1c	4.97 $\pm$ 0.08c	149a	0.26 $\pm$ 0.02b	75.8 $\pm$ 1.22b
Brackish	3.7 $\pm$ 0.33c	6.96 $\pm$ 0.15b	-266c	0.22 $\pm$ 0.09b	80.1 $\pm$ 5.32b
Salt	25 $\pm$ 0.58b	7.50 $\pm$ 0.08a	-103b	0.61 $\pm$ 0.06a	58 $\pm$ 2.43c
Mangrove	35.7 $\pm$ 1.76a	6.93 $\pm$ 0.01b	-39b	0.58 $\pm$ 0.09a	58 $\pm$ 0.61c

Pilot Study Table B-2. Mean ( $\pm$ standard error) of soil organic matter, carbon (C) and nitrogen (N) properties at 0 to 5cm depth along the salinity gradient at different marsh sites (freshwater, intermediate, brackish, salt and mangrove) for the pilot study in August 2015. Different lowercase letters represent significant differences between site means using Tukey's HSD test at  $\alpha=0.05$ .

Marsh site	Organic matter (%)	Organic C (%)	Total N (%)	C:N	Extractable NO <sub>3</sub> <sup>-</sup> ( $\mu$ mol g <sup>-1</sup> )
Freshwater	67.6 $\pm$ 2.26a	33.9 $\pm$ 0.78a	2.5 $\pm$ 0.15a	15.8 $\pm$ 0.9a	0.63 $\pm$ 0.21a
Intermediate	33.5 $\pm$ 1.82b	14.8 $\pm$ 1.57b	1 $\pm$ 0.15b	17.3 $\pm$ 0.54a	0.23 $\pm$ 0.03ab
Brackish	29.3 $\pm$ 5.39b	15.3 $\pm$ 4b	1 $\pm$ 0.23b	17.5 $\pm$ 0.82a	0.26 $\pm$ 0.04ab
Salt	9.17 $\pm$ 0.5c	4.2 $\pm$ 0.55c	0.3 $\pm$ 0.03c	15.6 $\pm$ 0.99a	0.12 $\pm$ 0.01b
Mangrove	12.3 $\pm$ 0.97c	5.4 $\pm$ 0.6c	0.4 $\pm$ 0.05c	17.7 $\pm$ 1.12a	0.13 $\pm$ 0.01b

Pilot Study Table B-3. Linear regressions between soil properties and CO<sub>2</sub> production (nmol gdw<sup>-1</sup> day<sup>-1</sup>) in August 2015.

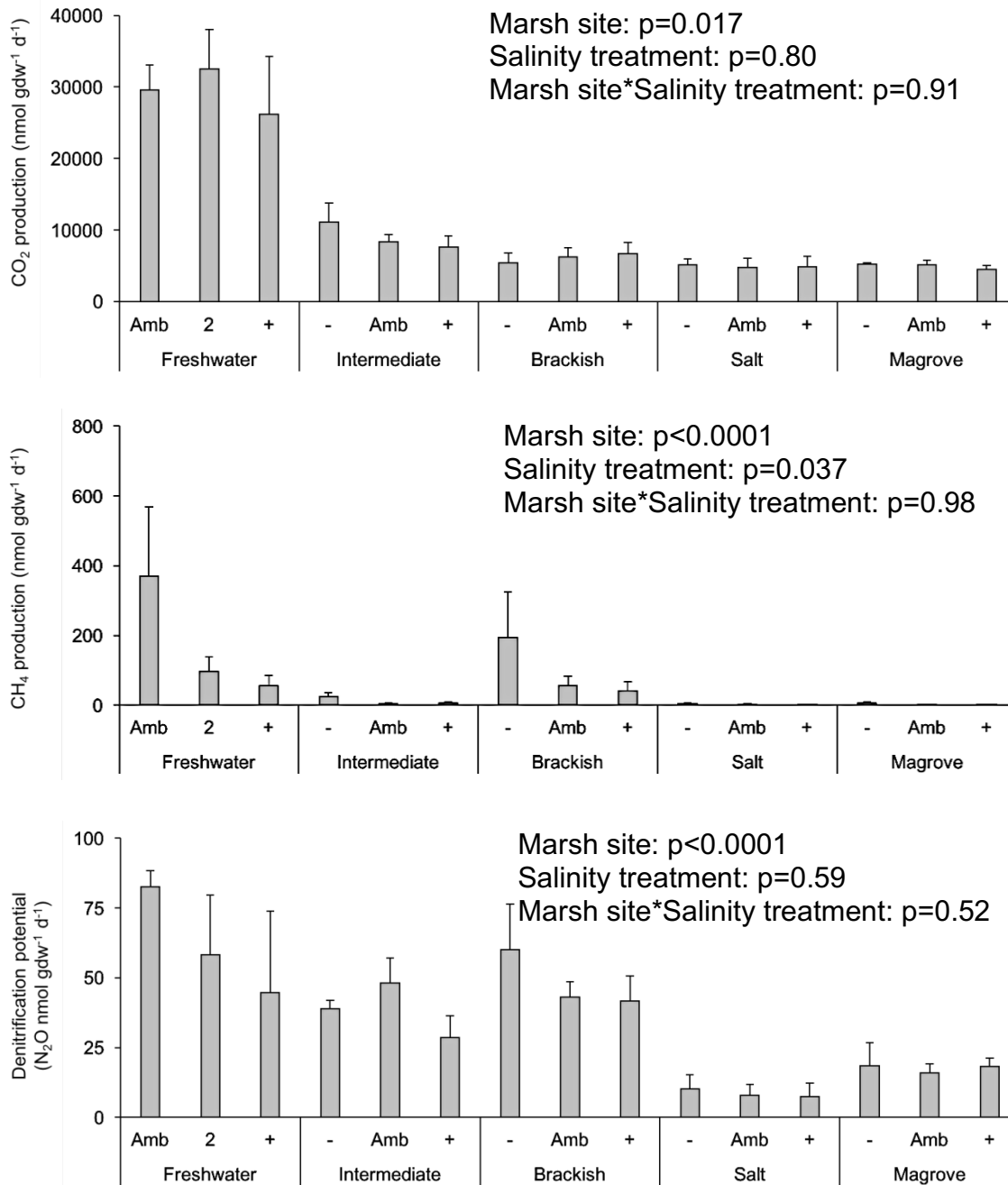
Variable (X)	Regression equation	r <sup>2</sup>	p-value
pH	1/CO <sub>2</sub> = -0.0002 + 6.1x10 <sup>-5</sup> X	0.48	0.004
Bulk density (g cm <sup>-3</sup> )	1/CO <sub>2</sub> = 4.5x10 <sup>-5</sup> + 0.0003X	0.53	0.002
Organic matter (%)	1/CO <sub>2</sub> = 0.0003 – 3.3x10 <sup>-6</sup> X	0.61	<0.001
Organic carbon (%)	1/CO <sub>2</sub> = 0.0003 – 6.7x10 <sup>-6</sup> X	0.64	<0.001
Organic nitrogen (%)	1/CO <sub>2</sub> = 0.0002 – 8.8x10 <sup>-5</sup> X	0.61	0.001
Extractable NO <sub>3</sub> <sup>-</sup> (μmol N gdw <sup>-1</sup> )	1/CO <sub>2</sub> = 0.0002 – 0.0002X	0.33	0.024
Porewater salinity (psu)	1/CO <sub>2</sub> =10.0x10 <sup>-5</sup> + 4.0x10 <sup>-6</sup> X	0.38	0.019

Pilot Study Table B-4. Linear regressions between soil properties and CH<sub>4</sub> production (nmol gdw<sup>-1</sup> day<sup>-1</sup>) in August 2015.

Variable (X)	Regression equation	r <sup>2</sup>	p-value
Bulk density (g cm <sup>-3</sup> )	ln CH <sub>4</sub> = 5.3 – 8.2X	0.71	<0.001
Organic matter (%)	ln CH <sub>4</sub> = -0.11 + 0.08X	0.70	<0.001
Organic carbon (%)	ln CH <sub>4</sub> = -0.19 + 0.17X	0.79	<0.001
Organic nitrogen (%)	ln CH <sub>4</sub> = 0.004 + 2.14X	0.73	<0.001
Extractable NO <sub>3</sub> <sup>-</sup> (μmol N gdw <sup>-1</sup> )	ln CH <sub>4</sub> = 1.01 + 4.96X	0.31	0.031
Porewater salinity (psu)	ln CH <sub>4</sub> = 4.1 – 0.12X	0.62	<0.001

Pilot Study Table B-5. Linear regressions between soil properties and denitrification potential (N<sub>2</sub>O nmol gdw<sup>-1</sup> day<sup>-1</sup>) in August 2015.

Variable (X)	Regression equation	r <sup>2</sup>	p-value
pH	ln N <sub>2</sub> O= 3.6 – 0.5X	0.61	<0.001
Bulk density (g cm <sup>-3</sup> )	ln N <sub>2</sub> O= 1.4 – 2.8X	0.79	<0.001
Organic matter (%)	ln N <sub>2</sub> O= -0.4 + 0.03X	0.75	<0.001
Organic carbon (%)	ln N <sub>2</sub> O= -0.4 + 0.05X	0.70	<0.001
Organic nitrogen (%)	ln N <sub>2</sub> O= -0.3 + 0.7X	0.67	<0.001
Extractable NO <sub>3</sub> <sup>-</sup> (μmol N gdw <sup>-1</sup> )	ln N <sub>2</sub> O= 17.4 + 80.4X	0.45	0.006
Porewater salinity (psu)	ln N <sub>2</sub> O= 0.97 – 0.04X	0.84	<0.001



Pilot Study Figure B-2. Mean (+standard error) of CO<sub>2</sub> production, CH<sub>4</sub> production, and denitrification potential at five different marsh sites (freshwater, intermediate, brackish, salt and mangrove) in August 2015 ( $p<0.001$ ) exposed to different salinity treatments. “Amb” = ambient, “2” = 2psu, “-” = -5 psu from ambient salinity, “+” = +5 psu from ambient salinity.

## **VITA**

Natalie Ceresnak was born and raised in Nyack, New York and became inspired to pursue a degree and career in environmental sciences. She attended the University of Scranton in Scranton, Pennsylvania in 2011 and worked in the laboratory of Dr. Robert Smith, researching the effects of climate on bird migration. She graduated with a Bachelor of Science in Environmental Science in May 2015. After graduation, she moved to Baton Rouge, Louisiana to enter the Master's program at Louisiana State University (LSU) in the Department of Oceanography and Coastal Sciences (DOCS). She conducted her thesis research in wetland biogeochemistry under the co-advisement of Dr. Brian Roberts and Dr. R. Eugene Turner at the Louisiana Universities Marine Consortium (LUMCON). During her time at LSU and LUMCON, she was given the opportunities to attend and participate in several seminars and conferences. Her academic and research pursuits have enriched her scientific competence and prepared her for a future career in environmental sciences.